"Immunohistochemical expression of KRAS and BRAF in colorectal adenocarcinoma: Study at tertiary care centre from central India"

Thesis

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Pathology and Lab Medicine

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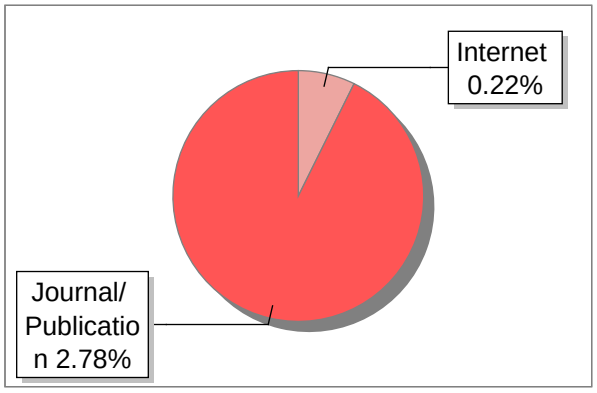

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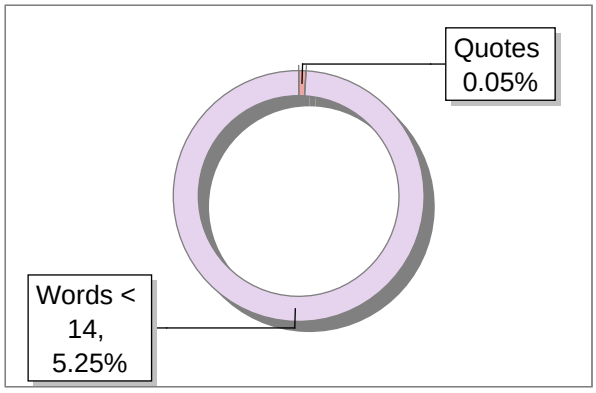

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INDEX

Sl. No.	Section	Page number
1	Introduction	1-3
2	Review of literature	4-31
3	Aims and objectives	32
4	Materials and methods	33-42
5	Results	43-79
6	Discussion	80-94
7	Summary	95-97
8	Conclusion	98
9	Strengths of the study	99
10	Limitations of the study	100
11	References	101-113
12	Patient case record	114-115
13	Participant information sheet	116-120
14	Informed consent form	121-123
15	Master chart	124-125

INDEX OF TABLES

Table 1: IHC interpretation protocol for KRAS in colorectal adenocarcinoma…….……..20

Table 2. BRAF IHC interpretation protocol for colorectal carcinoma …….…...25

Table 3. IHC interpretation protocol for KRAS in colorectal adenocarcinoma……...42

Table 4. BRAF IHC interpretation protocol for colorectal carcinoma in ……..42

Table 5. Summary of different types of specimens received …….43

Table 6. Table summarising different types of specimens received based on sex of the patients …44

Table 7. Various sites of colorectal adenocarcinoma (Including biopsy and resection cases) ranging from ileocaecal junction to anal verge.…….47

Table 8. Categorisation of the histological types seen in surgical resection and biopsy cases in total ……..48

Table 9. Showing the extent to tumor invasion ……...55

Table 10. Average number of tumor buds ……...57

Table 11. Number of cases with lymphovascular invasion ……...…59

Table 12. Lymph node status (Positive and Negative) and Tumor bud score ……...…….59

Table 13. Grading of KRAS expression in biopsy and surgical resection cases ……..61

Table 14. The table summarises the number of cases based on histological grade and Grade of KRAS expression ….64

Table 15. KRAS expression and Lymphovascular invasion ……..65

Table 16. KRAS expression and Tumor bud score ……..….66

Table 17. Association between KRAS grade and Tumor bud score ……..…….67

Table 18. Association between KRAS grade and T staging …….68

Table 19: Association between KRAS immunohistochemical expression and Node staging ……...……69

Table 20. Grading of BRAF expression in biopsy and surgical resection cases ……..……71

Table 21. BRAF immunohistochemical expression and Histological grade ……..…….74

Table 22. BRAF immunohistochemical expression and Lymphovascular invasion ……...…..75

Table 23. Grading of BRAF IHC and Tumor bud score ……..……..76

Table 24. Association between Grading of BRAF expression and T staging.....77

Table 25. Association between BRAF expression and Lymph node staging ……..……..78

Table 26. Association between both KRAS and BRAF positive and negative cases ……..…79

Table 27. Comparison of mean age and sex of the patients.……..80

Table 28. Comparison of size histological type and histological grade of Colorectal adenocarcinoma ……82

Table 29. Comparison of tumor size and lymphovascular invasion ……...83

Table 30. Comparison of Tumor bud score ……...84

Table 31. Comparison of various methods of KRAS mutation ……..……..85

Table 32. Association between Tumor staging and KRAS mutation ……..…87

Table 33. Comparison between KRAS mutation and tumor bud score.……..88

Table 34. Comparison between KRAS mutation and lymphovascular invasion ……88

Table 35. BRAF mutation in colon cancer by various methods

……...90

Table 36. Comparison between BRAF mutation and Tumor staging

……...91

Table 37. Comparison between BRAF mutation and Lymphovascular invasion

……...…92

Table 38. Comparison between BRAF mutation and Tumor staging.……..93

Table 39. Comparison between cases with both KRAS and BRAF mutation……...…94

INDEX OF FIGURES

Figure 1. Figure showing the main pathway includes APC mutation in the initial stage from the progression of normal colorectal epithelium to adenoma. Later on, KRAS and BRAF mutations occur. The last mutation to occur is the loss of TP53……..……..……11

Figure 2. Figure showing the normal pathway shows factors affecting normal cell growth and stimulation of cancer cells due to BRAF mutation ……...…….22

Figure 3. Shows gross specimen of Right hemicolectomy specimen ……35

Figure 4. Shows Abdominoperineal resection specimen ……36

Figure 5. Figure showing The automated immuno-stainer (VENTANA BenchMarkXT) ……37

Figure 6. Showing normal crypts of intestine staining positive for KRAS taken as positive control……38

Figure 7. Showing Seminiferous tubules of testis staining positive for BRAF taken as positive control ……...…...39

Figure 8. The bar graph shows age wise categorisation of biopsy and surgical resection specimens……...….....45

Figure 9. Pie chart describes the distribution of the site of tumor …….46

Figure 10. Shows Adenocarcinoma Colon where the tumor is arranged in Glandular architecture ……49

Figure 11: Shows Mucinous adenocarcinoma where the mucinous component is > 50% and also the tumor cells are seen floating the extracellular mucin pools...50

Figure 12. Shows Signet cell Carcinoma ……..51

Figure 13. Shows Mixed neuroendocrine and non-neuroendocrine tumor with adenocarcinoma component……..……..52

Figure 14. Pie chart showing the number of cases received and their histological grade categorised in Well, Moderate and Poorly Differentiated. ……...……53

Figure 15. Shows Well-differentiated Adenocarcinoma A) and B) H and E stain at 10 X and 20X respectively show tumor arranged predominantly (> 95%) in glandular pattern. ……..54

Figure 16. Shows Moderately-differentiated Adenocarcinoma. A) and B) H and E stain at 10 X and 20X respectively show tumor arranged in glandular pattern (50-95%) and also in sheets and trabeculae.

……54

Figure 17. Comparison between tumor size and Lymph node staging……....56

Figure 18. Shows Low tumor bud (0-4) A) and B) The images show no tumor buds at the advancing end of the tumor (H and E 10X and 20X respectively).

……..57

Figure 19. Show Intermediate tumor buds (5-9). A) and B)The image shows few tumor buds at the advancing end of the tumor (H and E 10X and 20X respectively)

…….58

Figure 20. Shows High (>10 tumor buds) A) and B)The image shows no tumor buds at the advancing end of the tumor (H and E 10X and 20X respectively…….…….58

Figure 21. Total number of cases which were positive for KRAS (including biopsy and resection cases)……..…….……60

Figure 22 Shows KRAS immunostaining (Grade 0) where no cells show staining; A) and B); 10X and 20X respectively…….……..61

Figure 23. Shows KRAS immunostaining (Grade 1) where 11-30% cells show weak intensity staining; A) 10X & B) 20X respectively

…….. 62

Figure 24. Shows KRAS immunostaining (Grade 2) where 31-50% cells show moderate intensity staining; A) 10X & B) 20X respectively …….……..62

Figure 25 Shows KRAS immunostaining (Grade 3) where >50% cells show strong intensity staining; A) 10X & B) 20X respectively

……..63

Figure 26: Showing BRAF immunostaining of biopsy and surgical resection cases ……...……70

Figure 27. Showing BRAF immunostaining of Grade 0 where no cells show positive staining; A) 10X & B) 20X respectively……71

Figure 28. Showing BRAF immunostaining of Grade 1 where weak cytoplasmic staining is seen in 10-33 % of cells; A) 10X & B) 20X respectively ……..72

Figure 29: Showing BRAF immunostaining of Grade 2 where moderate intensity cytoplasmic staining is seen in 33-66 % of cells; A) 10X & B) 20X respectively ……...……72

Figure 30. Showing BRAF immunostaining of Grade 3 where strong intensity cytoplasmic staining is seen in > 66 % of cells; A) 10X & B) 20X respectively ……...…….73

Abbreviations

APC: Adenomatous polyposis coli

BRAF: B Rapidly accelerated fibrosarcoma

CAP: College of American Pathologists

CK: Cytokeratin

CIN: Chromosomal instability

CRC: Colorectal carcinoma

DAB: 3-3’ diaminobenzidine

DNA: Deoxy ribonucleic acid

DPX: Dibutylphthalatepolystyrene xylene

EGFR: Epidermal growth factor receptor

ERK: Extracellular signal-regulated kinase

FAP: Familial adenomatous polyposis

FFPE: Formalin fixed paraffin embedded

GDP: Guanosine diphosphate

GTP: Guanosine triphosphate

H&E- Hematoxylin and Eosin

ITH: Intra-tumour heterogeneity

IHC: Immunohistochemistry

KRAS: Kirsten rat sarcoma

MAPK: Mitogen activated protein kinase

MEK: Mitogen activated protein kinase Extra cellular signal regulated kinase

MSI: Microsatellite instability

mRNA: messenger ribonucleic acid

NGS: Nest generation sequencing

PanIN: Pancreatic intraepithelial neoplasia

PBS: Phosphate buffered saline

PIK3CA: Phosphatidylinositol-4,5-bisphosphonate 3-kinase catalytic subunit alpha

PCR: Polymerase chain reaction

RNA: Ribonucleic acid

RAF: Rapidly accelerated fibrosarcoma

RAS: Rat sarcoma

TILs: Tumor infiltrating lymphocytes

TNM: Tumor Nodes Metastases

Introduction

‘Neoplasia, which means "new growth," refers to the group of cells and stroma that make up the new growth field (1).Colorectal cancer (CRC) comes third in terms of recognition and second in terms of mortality worldwide. It is estimated that by the year 2035, the total number of deaths from rectal and colon cancer will increase by 60% and 71.5%, respectively(2).Large and small bowel cancer incidence rates are low in India, and rectal cancer is more prevalent than colon cancer. In eight population registries, the incidence rates of colon cancer range from 3.7 to 0.7/100,000 for men and 3 to 0.4/100,000 for women(3).It is a disease that presents through a variable clinical course including weight loss, loss of appetite, per rectal bleeding, intestinal obstruction etc. and with significant inconsistency. CRC is presently the fourth leading cause of cancer-related death worldwide. In men it comes in second place behind lung cancer and for women comes as the third leading cause of cancer death after breast cancer.There is a wide variation towards the response to treatment, even within tumors with similar histopathological characteristics (4). Fifty percent of them will succumb within 5 years from diagnosis, usually as a result of metastatic disease (5).Although global mortality is decreasing, the incidence and mortality rate among younger patients (50 years old) is increasing(6).Distant metastasis is the main cause of death in CRC patients. However, curative resection is possible in <25% of patients with advanced-stage disease (7).

Various screening modalities are available for colorectal adenocarcinoma(8). These are:

- Fecal occult blood test
- Sigmoidoscopy
- Colonoscopy
- Virtual colonoscopy
- DNA stool test

The occurrence of colorectal cancer is caused by a variety of genetic changes, such as chromosomal abnormalities and epigenetic modifications affecting many genes that control cell division, differentiation, apoptosis, and angiogenesis (9).

The activation of oncogenes(KRAS and BRAF) as well as the inactivation of tumour suppressor genes (APC, p16 and p53) and mismatch repair genes, including MLH1 and MSH2 and, to a lesser extent, PMS2 and hMSH6, is now widely acknowledged as the primary mechanisms by which sporadic colorectal cancers frequently develop from preneoplastic lesions (10).

KRAS is a proto-oncogene that produces a tiny 21-kD protein that binds guanosine triphosphate (GTP) and guanosine diphosphate (GDP), which is used to control how cells react to various external stimuli(11). Wild-type KRAS binds to GTP to become active, and this conformational change enables the protein to bind and activate more than 20 recognised downstream effectors, including Raf, BRAF, mTOR, MEK1 and 2, ERK, AKT, and PIK3CA. These downstream effectors have a variety of distinct effects, such as the inhibition of apoptosis, stimulation of cell growth, cell transformation, angiogenesis, migration, and differentiation(12).

BRAF is a serine/threonine protein kinase that participates in the mitogen-activated protein kinase (MAPK) pathway, which regulates cell proliferation, differentiation, migration, survival, and angiogenesis. Many cases of cancer are caused by dysregulation of this system. The RAS small guanidine triphosphatase (GTPase), which activates the RAF family proteins (ARAF, BRAF, and CRAF, commonly known as RAF1), is a component of this pathway. Activated RAF proteins cause MEK1/2 proteins to be phosphorylated and activated, which in turn causes ERKs to be phosphorylated and activated. Several transcription factors are among the many substrates that ERKs phosphorylate, and they also control a number of important cellular processes(13).

Treatment options for metastatic disease have changed in recent years with the introduction of targeted therapies including anti-epidermal growth factor receptor (EGFR) therapies targeting EGFR transduction cascade(14). Cetuximab and panitumumab are anti-EGFR monoclonal antibodies designed to disrupt the EGFR signaling pathway at the EGFR receptor's extracellular region. These two medicines are utilized in the treatment of metastatic CRC as single agents or in conjunction with standard chemotherapy(15).

Not many studies, particularly from central India, have been published in journals demonstrating the immunohistochemistry expression of KRAS and BRAF in colorectal carcinomas.

In this study, wehave performedimmunohistochemistry to examine KRAS and BRAF expression in colorectal adenocarcinoma and assessed the significance of KRAS and BRAF expression in relation to histo-pathological features like tumour size, histological type and grade of tumour lympho-vascular invasion, tumour budding, and stage (TNM stage).

Review of literature

Colorectal cancer prognosis prediction is critical for selecting treatment options. In this regard, histopathological factors continue to be important. Factors influencing prognosis include tumour size, histological type and subtype, the existence of signet ring morphology and the degree of differentiation, as well as the presence of lympho-vascular invasion and lymph node involvement(16). The following review of literature pertains to the significance of risk factors and histopathological features in colorectal adenocarcinoma.

Risk factors

As a disease that is thought to be diverse, the incidence of CRC is connected with a number of risk factors. In contrast to other risk factors, which only slightly enhance the risk of CRC (24), genetic and environmental factors both significantly contribute to the aetiology of CRC and raise its risk compared to others(17, 24).

Inherited genetic risk: One of the uncontrollable CRC risk factors is hereditary factors. The risk of CRC is raised by a number of genetic CRC syndromes. Familial adenomatous polyposis and Hereditary non polyposis carcinoma colon, sometimes referred to as Lynch syndrome , are the most prevalent of these disorders. These two hereditary diseases account for around 5% of all instances of CRC(18).

Sex: In comparison to women, men had a slightly increased risk of developing CRC. Men are 1.5 times more likely than women to acquire CRC, regardless of age or location. Additionally, women are more prone to right-sided colon cancer than males are, and this variant has a more aggressive phenotype than left-sided colon cancer(19).

Age: Another uncontrollable CRC risk factor is age. Individuals under the age of 50 years account for more than 90% of CRC cases. People aged 60 to 79 years had a >50-fold greater incidence rate of CRC than people under the age of 40(20).

Environmental and lifestyle factors: Strong correlations between environmental and lifestyle variables and the development of CRC have been found. The risk of CRC is heightened by low socioeconomic position and inadequate medical care. rich meat consumption, red and processed meat consumption, diets rich in fat, and diets deficient in fruit, vegetables, and fibre are all nutritional habits linked to an increased risk of CRC. Obesity, excess body weight, and inadequate physical activity have also been linked to a higher risk of CRC. An increased risk of developing CRC may also be linked to heavy drinking and cigarette smoking(21).

Histopathological aspects of Colorectal adenocarcinoma

Adenocarcinomas, which develop from the epithelial cells of the colon mucosa, account for more than 90% of colorectal cancers. Neuroendocrine, squamous cell, adenosquamous, spindle cell, and undifferentiated carcinomas are further uncommon forms of colorectal cancer. The hallmark of conventional adenocarcinoma, which serves as the foundation for histologic tumour grading, is glandular development. More than 95% of the tumour in a well-differentiated adenocarcinoma is gland-forming. Adenocarcinoma with moderate differentiation exhibits 50–95% gland development. Poorly differentiated adenocarcinoma has about 50% gland development but is primarily solid. Approximately 70% of colorectal adenocarcinomas are identified as moderately differentiated in actual practice(22).

Numerous studies have shown that a two-tiered grading method that classifies poorly differentiated as high grade (less than 50% gland development) and well and moderately differentiated as low grade (50%) minimises interobserver variation and enhances predictive relevance(23). Multiple investigations have shown that a two-tiered grading method that classifies poorly differentiated as high grade (less than 50% gland development) and well and moderately differentiated as low grade (50%) minimises interobserver variation and enhances predictive relevance(24).

Special histological variants

Mucinous adenocarcinoma: Extracellular mucin makes up more than 50% of the tumour volume in this specific kind of colorectal cancer. Adenocarcinoma with mucinous features or mucinous differentiation are the standard terms used to describe tumours that have a significant mucinous component (>10% but <50%)(25). Many mucinous adenocarcinomas are seen in patients with hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome) and thus have high-level MSI (MSI-H) tumors(26).

Signet ring cell adenocarcinoma: Similar to mucinous carcinoma, signet ring cell carcinoma is identified by the presence of more than 50% of tumour cells exhibiting these characteristics, which include a protruding intracytoplasmic mucin vacuole that pushes the nucleus to the periphery(27).

Medullary carcinoma: The epithelioid neoplastic cells that make up this tumour have sheets of sizeable vesicular nuclei, noticeable nucleoli, and an abundance of cytoplasm. On resection specimens, it typically has a pushing border and is characterised by clearly visible lymphocytes that have invaded the tumour(28).

Immunohistochemical phenotype: Cytokeratin (CK) 20, CK7, and CDX2 are the most frequently used immunohistochemical markers for colorectal cancer. Positive staining for CK20 and negative staining for CK7, which is a relatively specific staining pattern for colorectal origin, is the most prevalent immunophenotype of colorectal adenocarcinoma(29)(30).

Precursor lesions:

Adenomas: Adenomas are by definition clonal lesions that exhibit at least low grade dysplasia and are distinguished by enlarged, hyperchromatic, and elongated (pencillate) nuclei organised in a stratified arrangement along the basement membrane. Apoptosis activity may be elevated and mucin depletion may be present in the adenomatous cells. It is interesting to note that adenomatous polyps seem to form using a "top-down" approach. The top-down approach says that the stem cells are located near the base of crypts in the colon. However, dysplastic cells are frequently observed at the luminal surface of the crypts in the early stages of carcinogenesis, although the cells at the bases of these same crypts seem morphologically normal. The genetic abnormalities of adenomatous polyposis coli (APC) and neoplasia-associated gene expression patterns were frequently seen in the dysplastic cells at the apices of the crypts. Cells near the base of these same crypts, however, did not have these modifications and were not clonally connected to the adjacent altered cells above them. These findings suggest that a top-down process governs the development of adenomatous polyps. Genetically modified cells in the surface mucosae travel laterally and downward to create new crypts, which first connect to already healthy crypts before eventually taking their place(31).

Serrated polyps:

Any polyp that exhibits a serrated (sawtooth or stellate) architecture of the epithelial compartment is referred to as a serrated polyp. It is a diverse group of lesions that mostly consists of classic serrated adenoma, sessile serrated adenoma/polyp, and hyperplastic polyp. Hyperplastic polyps are the most common serrated lesions that are likely to be found in the distal colon and are small in size (<5 mm) (32). In terms of histology, These are distinguished by a straightforward tubular architecture with elongated, straight crypts, luminal serration that is more pronounced in the higher regions of the crypts, and an apparent surface maturation. The basal region of the crypts, which is still thin and non-serrated, is the only area where proliferation can occur(33).

Dysplasia in inflammatory bowel disease:

A well-known risk factor for the emergence of dysplasia and cancer is inflammatory bowel disease. In this context, dysplastic lesions can be elevated or flat (endoscopically undetectable), and both are categorised as either indeterminate for dysplasia, low-grade dysplasia, or high-grade dysplasia. Raised lesions are sometimes referred to as dysplasia-associated lesions or masses, and they can be hard to tell apart from spontaneous adenomas (34).

Familial adenomatous polyposis:

The Familial adenomatous polyposis (FAP)is caused by a germline mutation in the APC gene, on chromosome 5q21.The early development of hundreds to thousands of adenomatous polyps in the colorectum is a hallmark of the uncommon familial adenomatous polyposis, an inherited autosomal dominant colorectal cancer condition. Associated features include upper gastrointestinal tract polyps, congenital hypertrophy of the retinal pigment epithelium, desmoid tumors, and other extracolonic malignancies. At a typical age of 35 to 40 years, colorectal cancer almost always progresses if untreated (35).

Peutz-Jeghers syndrome:

Hamartomatous polyps in the gastrointestinal tract, pigmented mucocutaneous lesions, and a higher risk of gastrointestinal and extragastrointestinal malignancies are all features of this autosomal dominant hereditary cancer syndrome. (36) The lifelong cumulative risk of colorectal cancer is close to 40%(37).

Juvenile polyposis syndrome:

This is an autosomal dominant inherited cancer syndrome. A diagnosis of this condition is made if a person has (I) five juvenile polyps in the colorectum, (II) juvenile polyps throughout the digestive system, or (III) any number of juvenile polyps plus a family history of juvenile polyposis(38).In patients with juvenile polyposis syndrome, the cumulative lifetime risk of developing colorectal cancer is also close to 40%(39).

Malignant Polyp

A polyp that has an invasive adenocarcinoma in the submucosa is known as a malignant polyp. The state of the resection margin, histologic grade, and the presence or absence of lymphovascular invasion are a few crucial histologic aspects that must be evaluated when a malignant polyp is found. These variables affect the likelihood of unfavourable outcomes such as nodal metastasis and/or local recurrence after polypectomy (40).

Pathogenesis and Molecular Classification

Colorectal cancer is a diverse group of illnesses and has a unique genetic and epigenetic origin. Attempts have been made to categorise colorectal malignancies based on geography, histology, etiologic factors, and molecular processes of carcinogenesis in order to enhance therapeutic care and better predict patient outcomes(41).

Both the MSI (Microsatellite instability)and CIN (chromosomal instability) pathways describe the pathophysiology of colorectal cancer based on genetic abnormalities that result in the loss of tumour suppressor gene activity and/or the acquisition of oncogene function. Epigenetic instability has received a lot of attention recently, and it is now thought to be involved in the pathogenesis of roughly one-third of colorectal malignancies(42). Numerous epigenetic

modifications, such as DNA methylation, histone modifications, and chromatin remodelling, regulate gene expression in addition to DNA sequence and structure(43). One of the most thoroughly researched epigenetic modifications linked to colorectal cancer is the hypermethylation of genes' promoter regions, which silences genes (44). In both sporadic and syndromic colorectal tumours, the CIN pathway is involved. Chromosomal gains and losses as well as karyotypic aberrations, which can be detected through DNA ploidy or loss of heterozygosity tests, are characteristics of CIN tumours(41).

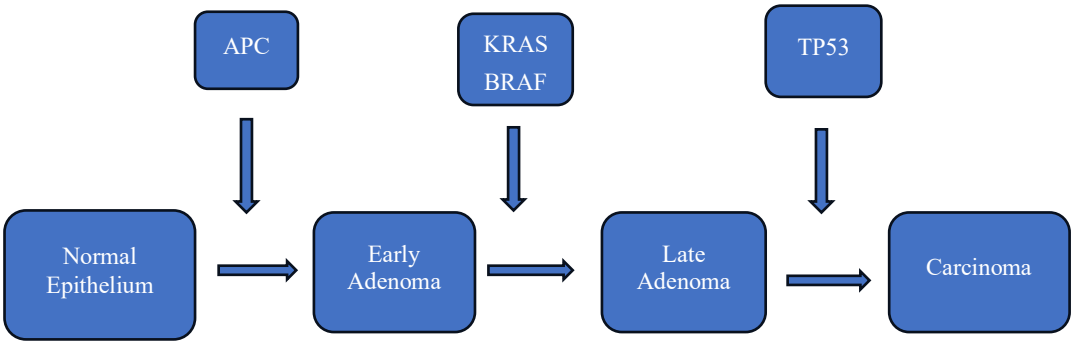

Figure 1 (Adapted from Nguyen et al) The main pathway includes APC mutation in the initial stage from the progression of normal colorectal epithelium to adenoma. Later on, KRAS and BRAF mutations occur. The last mutation to occur is the loss of TP53(45).

Molecular genetic testing

The recent work in cancer genomes has sparked a fundamental leap in our comprehension of the key molecular changes underlying tumour growth. Because of the development of extremely sensitive and accurate technical platforms and the growing body of information brought on by concerted international efforts, better medications may now be produced(46).

The very high computing power and very powerful tools of DNA and RNA sequencing have armed us to analyse a large set of database and do genetic profiling of a large number of cancers. These efforts have led to identifying the genetics and epigenetics (gene mutation, promoter methylation, expression of proteins etc.) of many cancers. To date, many core genetic alterations behind carcinogenesis have been identified (47).

Tumor budding and its significance:

Tumour budding in colorectal cancer is connected with tumour progression and serves as an additional prognostic feature in the TNM classification. Tumour buds can be observed at the invasive front and in the tumour centre of initial tumours. Tumour budding is linked to tumour progression, as well as local and distant metastases(48). The International Tumour Budding Consensus Conference established consensus guidelines. These recommendations are included in the most recent Colorectal Cancer Protocol from the College of American Pathologists (CAPs) (49).Tumor budding is graded using a three-tier system; scores of 0–4 indicate low budding, 5–9 indicate intermediate budding, and ≥ 10 indicate high budding(50).

Tumor-infiltrating Lymphocytes(TILs):

The presence of increased tumour-infiltrating lymphocytes is established as a positive prognostic factor in many malignancies including colorectal carcinoma. Previously it was focused on the relationship with microsatellite unstable (MSI) tumours, frequently as a marker to indicate which individuals should be screened for Lynch syndrome further.5 Recently, the focus has switched to the prognostic usefulness of TILs in all CRCs, regardless of mismatch repair gene status. However, multiple different approaches have been used to assess the TILs. The KlintrupMakinen Score is a widely used system in which the immune infiltrate is scored from 0 to 3.

Score 0- indicating no increase in inflammatory cells,

Score 1-A patchy increase in inflammatory cells at the invasive margin but no destruction of invading cancer cell islets (tumour cell nests or glandular structures),

Score 2 - A band-like infiltrate at the invasive margin with some destruction of cancer cell islets,

Score 3- A very prominent inflammatory reaction, forming a cup-like zone at the invasive margin, and frequent and invariable destruction of cancer cell islets(51)(52).

Recent advances in molecular genetics:

The "Big Bang" model says that in tumours which grow as a single expansion, produces countless intermixed sub-clones that are not subject to harsh selection, and in which both public (clonal) and most detectable private (subclonal) changes emerge early during growth. A genomic analysis of 349 individual glands from 15 colorectal tumours indicated the absence of selective sweeps, universally significant intra-tumour heterogeneity (ITH), and sub-clone mixing in remote locations. It also confirmed the assumption that the majority of detectable ITH arises from early private changes rather than later clonal expansions, revealing the profile of the primordial tumour. Furthermore, some tumours appear to be nasty from the start, with sub-clone mixing indicating early malignant potential. With major therapeutic consequences, this novel model provides a quantitative framework for interpreting tumour growth dynamics and the causes of ITH(53).

These efforts led to The Cancer Genome Atlas, an atlas of such novel genetic mutations enabling us to classify the tumors into different categories for treatment and to predict the clinical outcome of such treatment (54).

To date, a number of such targets are identified in different cancers viz. Her2neu and GATA3 in breast cancer, KRAS in pancreatic cancers, PIK3CA in small cell lung cancers and breast

carcinoma. In non- small cell lung cancers, about 60% of cases show a mutation in multiple driver oncogenes like EGFR, BRAF and KRAS.

The following pertains to the KRAS and BRAF genes, their structure, relation to the and their role in Colorectal adenocarcinoma and other malignancies along with different testing modalities of the mutations.

The KRAS gene

Polymorphism/Structure and Localization of the KRAS Gene:

A small GTPase transductor protein named KRAS is encoded by the KRAS gene, also known as the Kirsten rat sarcoma viral oncogene homolog (Ki-ras2). KRAS belongs to a group of small GTP-binding proteins, known as the RAS superfamily or RAS-like GTPases. KRAS is a member of the RAS superfamily, also referred to as the RAS-like GTPases, which is a subset of small GTP-binding proteins (55). More than 150 RAS-like genes have been found in the genomes of mammals(56). The most frequently studied proteins are those in the RAS subfamily (RAS, RHO, RAB, ARF, RAC, and RAN), including Harvey-RAS (H-RAS), neuroblastoma-RAS (N-RAS), and two variants of Kristen-RAS (K-RAS): the dominant form, known as KRAS4B, which is much more highly expressed in human cells. (55) KRAS's capacity to transmit outside signals to the cell nucleus makes it involved in the control of cell division. The switch from an inactive to an active form is regulated by intracellular signals. Once the GTP is bound to the KRAS protein, KRAS undergoes conformational changes that involve two regions of the protein, thus activating it(57).

KRAS contains four domains. The first domain includes 85 amino acids at the N-terminus and is identical in the three forms of RAS (KRAS, NRAS, and HRAS). The second domain contains

80 amino acids, with lower sequence identity (70–80%) among the three forms of RAS protein. These regions are important for the signalling function of the KRAS protein and jointly form the G-domain. The G-domain includes the GTP-binding pocket, where P-loop-phosphate binding loops interact with the b-phosphate and c-phosphate of GTP. This region plays an important role in the regulation of the biological activity of RAS protein(58).

'KRAS' Protein an Important Target to Study:

Wild-type KRAS usually promotes cell cycle progression, it can also induce growth arrest, apoptosis, and replicative senescence when increased to abnormal levels. This can be triggered by cellular stress, ultraviolet or ionizing irradiation, heat shock, and some cytokines. In these circumstances, triggering of growth arrest can represent a defence mechanism against inappropriate activation of RAS. (55) It has been demonstrated that the wild-type *KRAS* gene is a tumour suppressor that is frequently lost during tumour progression in many types of cancer. (59) Once the *KRAS* gene mutates, it acquires oncogenic properties and seems to be involved in the development of various human cancers (60).

Oncogenic mutations in the *KRAS* gene prevent the hydrolysis of GTP, thus permanently activating the RAS molecules. Overexpression of this mutated form of KRAS also inhibits glycosylation of the integrin β1-chain, resulting in altered polarisation and increased adhesiveness of colon cancer cells. Expression of a mutated *KRAS* gene in fibroblasts has been shown to augment metalloproteinase 2 expression in the matrix and enhance the invasion of cancer cells (61). Also, the expression of this oncogenic form of KRAS protein has been shown to be associated with upregulated carcinoembryonic antigen expression and disturbance of epithelial cell polarization (62).

KRAS in Oncogenesis:

Activating *KRAS* gene point mutations have been detected in many types of human tumours. Such oncogenic forms of the *KRAS* gene are prevalent in pancreatic carcinomas (>80%), colon carcinomas (40–50%), and lung carcinomas (30–50%), but are also present in biliary tract malignancies, endometrial cancer, cervical cancer, bladder cancer, liver cancer, myeloid leukaemia and breast cancer (11).

KRAS and different neoplasms:

Laghi et al.studied a series of pancreatic cancer cases with a small number of cases of pancreatic intraepithelial neoplasia(PanIN), a precursor disease, have been studied, and the results suggest that multiple K-RAS mutations may coexist in neoplastic pancreas. To ascertain if mutant-K-RAS polyclonality is a common or unusual feature of pancreatic carcinogenesis, they studied a collection of periampullary malignancies (41 pancreatic, 13 biliary, and two ampullary adenocarcinomas). Results show a high prevalence of various K-RAS mutations in both PC and biliary malignancies, and they imply that different methods may be contributed to tumour progression by distinct clonally heterogeneous PC precursor lesions(63).

Huncharek et al. presented the results of a meta-analysis done to ascertain the information already available on the function of KRAS mutations in NSCLC. They came to the conclusion that the KRAS mutation data may be related to reduced survival in NSCLC, but this conclusion has yet to be substantiated by well conducted multivariate studies, which should take into account the impact of recognised prognostic factors(64).

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to be substantiated by well conducted multivariate studies, which should take into account the impact of recognised prognostic factors(65).

Bazan et al. Exons 5 to 8 of the TP53 gene's mutation analyses revealed mutations in 43% (68/160) of the cases, whereas exon 2 of the KRAS gene's mutation investigations revealed mutations in 46% (74/160) of the cases. Multivariate analysis revealed that specific TP53 mutations or their combination with specific KRAS mutations at codon 13 were substantially linked with clinical outcomes. They came to the conclusion that particular KRAS mutations at codon 13 in combination with specific TP53 mutations in the L3 domain alone or both are linked to a worse prognosis in sporadic CRC(66).

Brink et al. In this study, 737 incident colorectal cancer patients from the Netherlands Cohort Study were examined in order to assess subgroups based on the presence of K-ras mutations. In contrast to individuals with proximal or distal colon tumours or patients with rectosigmoid tumours, they also discovered that patients with rectal tumours have relatively the highest frequency of K-ras mutations. The equally high prevalence of K-ras mutations in cases with and without a family history of colorectal cancer disproves the idea that this mutation plays a significant role in familial colorectal cancer and may instead suggest that K-ras mutations are more likely related to environmental mechanisms of colorectal carcinogenesis(67).

Methods of testing KRAS mutations:

There are several methods for detecting KRAS mutations, each with its own set of advantages and disadvantages. There is currently no clear guideline that gives a precise diagnostic algorithm. While clinical guidelines for KRAS mutational analysis are evolving, the American Society of Clinical Oncology currently recommends that all patients with stage IV colorectal carcinoma who are candidates for anti EGFR antibody therapy have their tumour tested for KRAS mutations.

Real-Time PCR

AS-PCR (allele-specific real-time polymerase chain reaction) is a well-known technique for detecting somatic mutations in KRAS. This method selectively amplifies wild-type or mutant DNA molecules. The benefits of this approach are its low cost, great sensitivity (1% of allele frequency is detected), and single closed tube performance (which prevents contamination). However, the application of AS-PCR in KRAS mutation detection is limited due to low discriminating effectiveness between mutant and wild-type DNA for a variety of mismatches. Another drawback is that only specific mutations can be identified (68).

Next generation sequencing

This modality allows simultaneous detection of multiple gene variants, to quantify the allelic frequency and analyze multiple genes. In one study, out of 468 CRC patient samples 77 tests were done by both standard techniques and Next generation sequencing (NGS). There were concordant results between testing methodologies in 74 out of 77 patients, or 96% (95% CI 89% to 99%). NGS may also lead to incorrect results and has not yet been established as a gold standard. There is an intrinsic error rate with different types of NGS, and adjusting analysis sensitivity too high will allow for the inclusion of false-positive mutations. Additionally, due to tumour heterogeneity, mutations can be present at varying frequencies within the tumour(69).

Immunohistochemical assay for detection of K-ras protein

Elsabah et al. studied K-ras protein expression by immunohistochemistry using Rap1A polyclonal antibody, paraffin-embedded tissue samples from 26 patients with metastatic

colorectal cancer were examined for K-ras protein expression by IHC. The subjective evaluation and correlation of staining patterns with clinicopathological characteristics was done. In 42.3% of cases, cytoplasmic positivity for K-ras was found. Out of these, 26.9% of cases had high positivity, while 15.4% had moderate positivity. Positivity was seen in 50% of moderately differentiated carcinomas and 38.9% of poorly differentiated carcinomas. Positive staining was found in 40% of individuals with positive lymph node metastases and 45.5% of cases without nodal metastasis (70).

Review of literature pertaining to criteria used to analyse the IHC staining pattern and their intensity for KRAS:

Several studies have been published for KRAS immunohistochemical reactivity in the percentage of positive cells, and staining intensity has been taken into account for result interpretation. We are discussing one of them in tabular form.

Table 1: IHC interpretation protocol for KRAS in colorectal adenocarcinoma (71)		
Staining grade	Intensity	Percentage of cells
0	Absent	0-10 % of cells
1	Weak	11-30%
2	Moderate	31-50%
3	Intense	>50%

Targeted therapy in KRAS mutated colorectal adenocarcinoma:

Although the mortality rate from the disease has decreased among people with early and localised disease because to advancements in treatment and early diagnosis, the prognosis for those with metastatic colorectal cancer remains grim. Making therapeutic decisions and enhancing survival outcomes require a thorough understanding of biological and genetic components. Understanding the mechanism and creating therapeutic targets for metastatic colorectal tumours with the KRAS mutation is one of these crucial steps. KRAS mutations are present in about 45% of patients with metastatic colorectal cancer. These mutations have a detrimental prognostic effect on overall survival in addition to carrying a predictive role for the lack of response to anti-EGFR therapy. Numerous investigations are being conducted to find compounds that can bind to the mutant KRAS sites or prevent the creation of the altered protein at the DNA level, hence preventing KRAS function (72). The first KRAS G12C specific, irreversible inhibitor to receive FDA approval is AMG 510 (Sotorasib). The KRAS is kept in an inactive, GDP-bound state by it (73). AMG 510 has demonstrated in preclinical trials to suppress ERK phosphorylation, a crucial downstream effector of KRAS, leading to a long-lasting full tumour shrinkage in mice (74). There are many more drugs like MRTX849 (adagrasib), MRTX1133, KR12 etc which target various mutations in KRAS and are under

preclinical trials for metastatic colorectal adenocarcinoma. The exact selection of the patients for cancer-directed therapy will be crucial to assure efficacy given our expanding knowledge of the heterogeneity of KRAS altered tumours and the variety of KRAS mutant forms.

KRAS mutation and resistance to anti EGFR therapy:

Many human epithelial malignancies, including head and neck squamous-cell carcinoma, CRC, breast, pancreatic, non-small cell lung, and brain cancer, have been found to express EGFR. Complex intracellular signalling pathways are stimulated by EGFR activation, and these pathways are closely regulated by the presence and identity of the ligand, the heterodimer composition, and the availability of phosphotyrosine-binding proteins. The PI3K-PTENAKT and RAS-RAF-MAPK pathways are the two main signalling pathways that EGFR activates(75). Evaluation of the impact of KRAS mutations mainly codon 12 is a mechanism of resistance to EGFR inhibition and a logical course of action given that KRAS plays a crucial role in the transduction of EGFR signalling. So studying the presence of KRAS mutation becomes mandatory when anti EGFR therapy is planned.

BRAF gene and MAP Kinase pathway:

BRAF is a proto-oncogene that is found on chromosome 7q37. This gene encodes a protein serine/threonine kinase that is involved in the RAS/RAF/MEK/ERK pathway. The epidermal growth factor receptor (EGFR) mediates the mitogen-activated protein kinase pathway (76). BRAF activates the MAP kinase pathway, which influences cell growth, proliferation, and differentiation, as well as other activities such as cell migration, apoptosis, and survival (77).

BRAF mutations have been found to be constitutively activated in 15% of all human malignancies. This gene is known to be altered in a number of sites, although the most common mutation is V600E(78).

The bulk of BRAF mutations result in the addition of new phosphomimetic residues or the deletion of the N-terminal region's auto-inhibitory confirmation, which facilitates kinase domain dimerization, a key process for kinase activity(79). ARAF, BRAF, and CRAF are the three different RAF gene types and their corresponding proteins. Historically, the first activating mutation in the BRAF gene, which was grouped in the kinase domain, was discovered in 2002 (78). There is a hydrophobic connection which is broken down by the BRAFV600E mutation, allowing BRAF kinase to fold into a catalytically active position and resulting in a 500-fold increase in kinase activity(80). Another mutation known as BRAFD594V mutation, often known as the "kinase-dead" version, has been linked to numerous malignancies. As a result of this kinase-dead B-Raf's interaction with C-Raf, oncogenic Ras genetic changes are potentiated, the MAPK signalling cascade is further stimulated, and tumour growth is subsequently accelerated (81).

BRAF Biochemistry:

The RAS-RAF-MEK-ERK1/2 signalling pathway includes the RAF protein. There are three different varieties of RAF proteins: A-Raf, B-Raf, and C-Raf. The BRAF protein is the only one with an oncogenic mutation. The 766 amino acids that make up the B-Raf protein are distributed across three conserved regions known as conserved regions 1, 2, and 3, respectively. The three conserved areas serve various purposes. The conserved regions 1 (CR1) and 2 (CR2) are serine-rich hinge regions, the conserved region 3 (CR3) is a catalytic region with serine/threonine protein kinase activity, and the conserved region 1 (CR1) is a RAS GTP

binding domain. This activity phosphorylates the consensus sequence on protein substrates (82).

The action of CR1 as a kinase inhibitor seizes after RAS-GTP binding, and CR2 functions as a hinge between CR1 and CR3, releasing CR1 from the auto-inhibitory CR1-CR3 connection. The dual-lobed CR3 structure has a bigger C-terminal lobe that binds to the substrate protein and a relatively smaller N-terminal lobe that binds to ATP. The kinase domain of the CR2 consists of 457–717 amino acids. The two lobes covering the active residue D576 in this kinase domain have an interior-facing active kinase cleft. The electrostatic interaction between the negatively charged phosphate group of ATP and the positively charged p-loop of the N terminal stabilises the binding of ATP to the N terminal (83).

Upon activation, B-Raf forms a dimer and is drawn to the plasma membrane where it is attached by a zinc finger motif made up of the amino acids 234–280 that binds phorbol ester. Raf proteins are differentially phosphorylated during this activation by the RAS-GTP complex, and B-Raf then exhibits serine/threonine-specific protein kinase activity (84).

BRAF:Pathway and RAS/RAFcascade:B-Raf is a well-known cytoplasmic serine-threonine protein kinase that belongs to the MAP kinase (MAPK) pathway and is triggered by several Ras activating receptors on the plasma membrane. Raf is recruited to the cell membrane by activated Ras, who then phosphorylates and activates MAP-ERK kinase (MEK), which in turn phosphorylates and activates extracellular signal-regulated kinase (ERK), the next element of the cascade. ERK is now phosphorylated and affects more than 150 downstream cytoplasmic and nuclear targets (85). They move into the nucleus and phosphorylate several transcription factors, including c-Myc, c-Jun, Ets, and c-Fos, among others, to operate on nuclear targets(86).

The cell cycle, proliferation, differentiation, and survival are now regulated by these transcription factors. Cell cycle proteins, apoptotic proteins like Bad and caspase 9, and

cytoskeletal proteins like paxillin and calnexin are all phosphorylated by ERK in the cytosol itself. distinct cell types have distinct overall effects. Due to the existence of several isoforms of each protein, including three isoforms of Raf, the regulation of this pathway is extremely complex. These proteins' isoforms, which are encoded by various genes, exhibit functional convergence and divergence. The fact that various isoforms can form homodimers with the same isoform and heterodimers with other isoforms adds to the complexity of this process. For instance, B-Raf can form heterodimers with C-Raf and homodimers with other B-Raf(87).

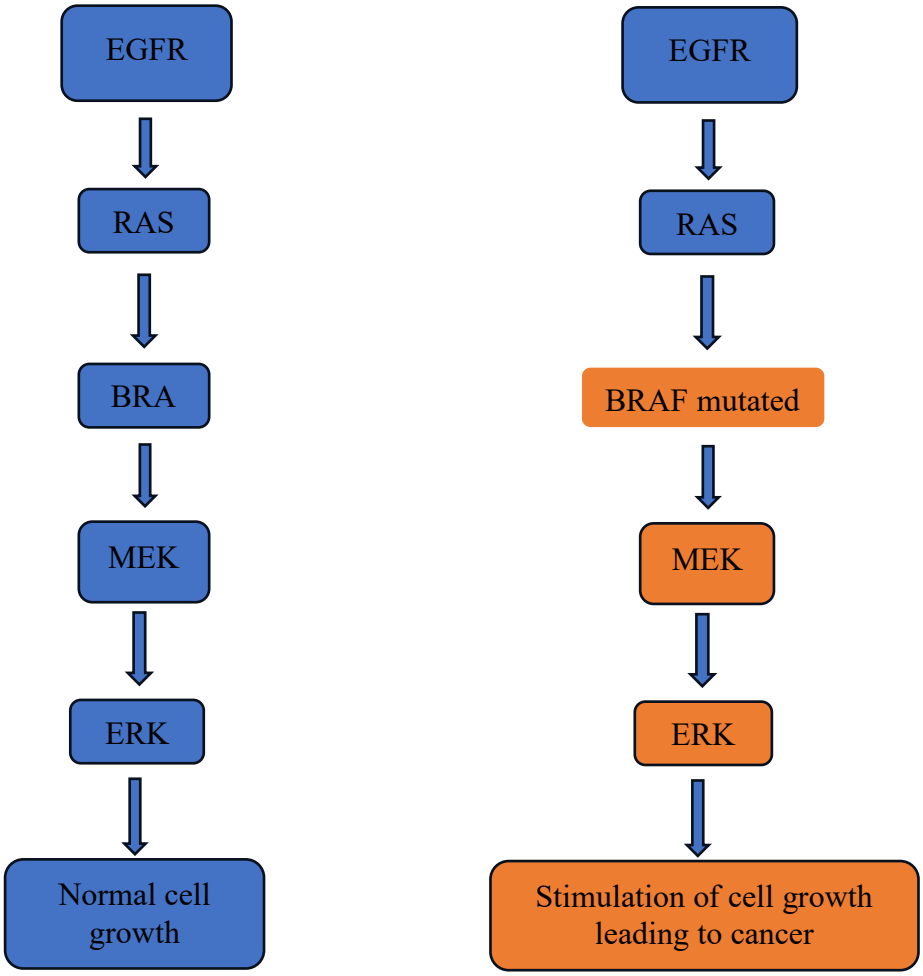

Figure 2 (Adapted from Zaman et al) The normal pathway shows factors affecting normal cell growth and stimulation of cancer cells due to BRAF mutation (46).

Type of mutations:

About 30 distinct BRAF mutations have been functionally characterised, and nearly 200 mutant BRAF alleles have been found(88). The three groups of mutations are based on how the mutation affects the activity of B-Raf. BRAF mutations classified as class 1 function as an active monomer and are independent of RAS. B-Raf from class 2 mutants functions as an active dimer.

Both of these two kinds of mutant B-Raf proteins largely separate themselves from the RAS GTPases, the upstream regulator. Due to the decoupling of B-Raf from RAS-GTPase, B-Raf becomes constitutively active, activating itself regardless of upstream cues for cell growth and division and promoting cancer(89). On the other hand, Class 3 mutant B-Raf proteins lack kinase activity but still require RAS- GTPase signalling to activate [38]. But as a result of some poorly understood mechanistic factors, they exhibit heightened MAPK signalling after RAS activation. Class 3 mutated BRAF activity depends on RAS-GTP activation, hence blocking upstream RAS signalling is an additional treatment approach that is not available for class 1 and class 2 BRAF mutated cancers (90).

BRAF and tumours:

The same BRAF mutation may be present in malignancies that differ histopathologically, such as hairy cell leukaemia, papillary thyroid carcinoma, melanoma, various CNS neoplasms, colon adenocarcinoma, Langerhans cell histiocytosis, and others. BRAF inhibitors, like as vemurafenib, can be used to treat these, which are collectively referred to as BRAF-omas.

BRAF mutations in CNS neoplasms:

Schindler et al.analysed that the majority of BRAFV600E mutations were found in WHO grade II pleomorphic xanthoastrocytomas (nearly 66% of cases), PXA with anaplasia (about 65% of cases), WHO grade III anaplastic gangliogliomas (about 50% of cases), WHO grade 1 gangliogliomas (about 18% of cases), and pilocytic astrocytomas (about 9% of cases) (91).

BARFV600E mutation in papillary thyroid carcinoma:

Yan et al. Using the medical records of 2048 patients with papillary thyroid cancer, a retrospective analysis examined the BRAFV600E status and examined the associations

between this mutation and various clinical outcomes and risk variables. Additionally, they discovered a connection between this mutation and variables including old age, high blood pressure, and Hashimoto's thyroiditis (92).

BRAF mutation in dermal / melanotic neoplasms:

Wysocka et al. discovered that nevi have higher rates of p16 and BRAFV600E mutations than melanoma. p16 expression was more frequently observed in the wild type of melanoma than the mutated type, while it was more frequent in mutated nevi than the wild type in p16-expressing nevi. A p16 mutation has a detrimental correlation with the rate of multiplication in melanoma cancer cells(93).

BRAF mutations in colorectal carcinomas:

Samowitz et al. studied a sizable number of colorectal cancer patients. They discovered that only 40, or 5%, of the 803 cases of microsatellite stable tumours had the BRAFV600E mutation. 43, or 51.8%, of the 83 tumours with microsatellite instability, or microsatellite instability, had this mutation. Additionally, they discovered that BRAFV600E mutations in tumours with microsatellite stability have a poor prognosis, whereas tumours with microsatellite instability, regardless of BRAF status, have a better prognosis (94).

Jacob R. Bledsoe et al. examined the sensitivity, specificity, and reliability of IHC in detecting the BRAF V600E mutation in cases of colorectal cancer that had undergone or been treated with chemotherapy, radiation treatment, or targeted therapy. They discovered that IHC has strong inter-observer consistency and is 96.3% sensitive and 98.5% specific for the BRAFV600E mutation testing, regardless of treatment status. They ran IHC on 20 samples both before and after treatment, and they discovered that both samples had perfect accuracy and agreement. Finally, they came to the conclusion that IHC is extremely reliable to identify

BRAF mutations in colorectal cancer, regardless of treatment status, site, or primary or metastatic tumour(95).

Methods of BRAF Mutation Testing:

There are several methods for finding BRAF mutations, and each method has advantages and disadvantages of its own. There is currently no set rule that offers a thorough diagnosis algorithm. IHC is sometimes utilised as the preferred screening method in general practice while molecular testing is employed for confirmation. In contrast, some facilities favour Real-time PCR (qPCR) or next-generation sequencing over IHC to identify mutations

Real-Time PCR (qPCR)

The BRAF mutation can be tested using qPCR, which amplifies initial tumour DNA sequences and tags them with labelled primers to find mutant and wild type sequences. To further determine the existence or absence of mutation, the strength of the wild type and mutant signals are contrasted. This method has a high degree of sensitivity and specificity while being reasonably quick, affordable, and efficient. The major drawback of this approach is the use of a small variety of primers that exclusively focus on particular mutations like V600E, K, and D. The Cobas ® 4800 and THxID ® assays have been authorised by the USFDA for use as confirmatory tests to find BRAF mutations.

BRAF mutation testing has recently been made possible by the development of the completely automated RT-PCR test IdyllaT M by Biocartis, which can identify BRAF V600E, E2, D, K, R, and M. The two advantages of this test are that it can detect just 1% mutant cells against a background of 99% wildtype cells on FFPE samples and that it is a very quick procedure since no DNA extraction step is necessary and instead, 5–10 m FFPE slices can be fed directly into the machine (96).

Sanger Sequencing

The gold standard method for testing acquired mutations used to be Sanger sequencing. This method allows us to find different kinds of BRAF mutations. However, this technique has some drawbacks, including the need for a significant percentage of mutant cells in the sample. (70) The sensitivity of this method was reported to be 98% while the specificity for BRAFV600E was 100% in a research involving 72 BRAF mutant samples. In order to test for the BRAFV600E mutation, this modality is employed as a confirming rather than a screening modality (97).

Next-Generation sequencing

This method enables the estimation of allelic frequency and the simultaneous analysis of numerous genes while simultaneously detecting multiple gene variations. One study with 72 BRAF-mutated samples indicated that the test's sensitivity and specificity were 98.6% and 100%, respectively. This modality's drawbacks include expense and time requirements. This method is very helpful for research and potential therapeutic targets because it offers information on additional driver variants (96).

Immunohistochemistry

We can identify the mutation because monoclonal antibodies attach to the mutant BRAFV600E protein. These days, many European institutes prefer using this strategy because it is simple, affordable, and reasonably quick way to test the mutation in FFPE tissue samples. Therefore, assuming it is later verified by molecular-based assays, IHC may be used as a screening tool of choice to discover mutant BRAF testing and start treatment. IHC can only detect the V600E mutation, hence instances with negative results from IHC should be checked for other BRAF variants using molecular methods (96).

Review of literature pertaining to criteria used to analyse the BRAF IHC staining pattern and their intensity in colorectal adenocarcinoma

Table 2: BRAF IHC interpretation protocol for colorectal carcinoma(98)		
Staining grading intensity	Staining Pattern	Interpretation of result
0	Absence of staining	Negative
1+	Weak cytoplasmic staining	Negative; Should go for molecular testing
2+	Moderatecytoplasmic staining	Positive
3+	Intense cytoplasmic staining	Positive

Targeted therapy in BRAF mutated colorectal adenocarcinoma:

Sorafenib, the first BRAF inhibitor, was created and clinically tested in melanomas; it is a broad-spectrum kinase inhibitor that targets VEGFR, PDGFR, CKIT, and FLT3 in addition to BRAF and CRAF. It was challenging to determine the best dose for this drug due to the lack of mutant specificity and the numerous targets for this molecule. Later a more mutant-specific vemurafenib was developed. Another mutant BRAF inhibitor that has received FDA approval is dabrafenib.

These RAF inhibitors alone have negligible to no impact on thyroid and colorectal cancers that harbour BRAF mutations. The paradoxical activation of the MAPK pathway following BRAF suppression, which causes cutaneous malignancies such squamous cell carcinoma, is the first of two restrictions on the use of anti-BRAF therapy. The emergence of resistance against BRAF monotherapy is the second restriction (99).

In order to get over these restrictions, BRAF-mutated or other MAPK pathway-driven tumours are being explored with MEK inhibitors such trametinib and MEK162. The advantage of utilising these inhibitors is that they do not paradoxically activate the MAPK pathway; however, their moderate efficiency is a drawback. As a result, researchers are looking into a combination therapy that uses BRAF inhibitors and anti-MEK inhibitors, which is working very well to treat these tumours(100). The treatment of colorectal cancer with the BRAFV600E mutation is currently being studied using this combination therapy with an anti-EGFR antibody (101).

3. AIM

To study the Immunohistochemical expression of KRAS and BRAF in colorectal adenocarcinomas in patients at the tertiary care center of AIIMS Bhopal, from central India

OBJECTIVES

1. Retrieving clinical, demographic and histopathological details of all patients diagnosed with Colorectal Adenocarcinoma between January 2018 – June 2023.
2. To study the immunohistochemical expression of KRAS and BRAF in colorectal adenocarcinoma and correlate it with histo-pathological features such as size of tumor, histological type and grade, lympho-vascular invasion, tumor budding and stage (TNM).

4. MATERIALS &METHODS:

The study was carried out on the available archived tissue samples available in the Department of Pathology and Lab Medicine, AIIMS Bhopal from January 2018 to June 2023

Cases and sample collection:

A total of 50 cases including 36 resection cases and 14biopsies were subjected to KRAS and BRAF mutation testing using IHC after preliminary evaluation of H&E slides. The study included FFPE blocks of cases diagnosed as colorectal adenocarcinoma on resection and biopsies having potential KRAS and BRAF mutations in viz.

The histological and routine IHC results were recorded as per the need of the study.

Sample collection:

Samples received between January 2018 to June 2023 in the Department of Pathology and Lab Medicine, AIIMS, Bhopal were included in the study.

Data were obtained from patients' records and requisition forms received in the Department of Pathology and Lab Medicine, AIIMS, Bhopal.

The patient's demographic as well as clinical details were noted. The patient's identifiers were not included.

Study design: Observational Cross sectional study

Study setting: Department of Pathology and Lab Medicine, AIIMS Bhopal; Department of General Surgery, AIIMS Bhopal; Department of Surgical Oncology, AIIMS Bhopal.

Study Period: The cases reported betweenJanuary 2018 to June 2023 were included in the study.

Sample size: A sample size of 50 cases including 36resections and 14 biopsies was taken.

Inclusion criteria: Patients diagnosed as colorectal adenocarcinoma on endoscopic biopsy and resected specimens during study period at Department of Pathology and Lab medicine at AIIMS Bhopal were be included.

Exclusion criteria for study participants: Patients for whom consent cannot be obtained, cases for which blocks are not available (as in the case of blocks issued to the patient), cases which are diagnosed as colorectal malignancies other than colorectal adenocarcinoma (lymphoma, neuroendocrine malignancies, mesenchymal neoplasm) and samples with inadequate tissue will be excluded.

Ethics approval: Ethics approval was obtained from the Institutional Human Ethics Committee for ethics approval. LOP No: IHEC-PGR/2021/PG/Jul/12 dated 28-02-2022.

Statistical Analysis: The data of the patients were collected and an excel sheet was prepared with the required parameters. The data were analysed in R Studio and analysis was done with R language.

- Mean and standard deviation was used for continuous variables. The distribution of continuous variables was assessed using Shapiro-Wilks test and subsequent analyses were done according to the distribution.
- Pearson's Chi-square and Fisher's exact were used for the comparison of categorical variables.
- Probability values up to 0.05 were considered significant.

Methodology:

The FFPE blocks of cases diagnosed as colorectal adenocarcinoma were retrieved and sections of 3 μm thickness were stained with hematoxylin and eosin.

Histopathological diagnosis was confirmed based on morphology for retrospective cases. For prospective cases, the diagnosis was made based on morphology by analysing the slides.

Histopathological features were noted including:

1. Histological type, grade, number of primary sites, tumor extent, lymphovascular and perineural invasion, number of tumor buds including tumor bud score, treatment effect if neoadjuvant chemotherapy was administered, number of positive regional lymph nodes and TNM staging.
2. The tumor buds were scored using a three-tier system.
    - Low (0-4)
    - Intermediate (5-9)
    - High (10 or more)

Grossing protocol of colorectal adenocarcinoma:

- Upon receiving the specimen, clinical details are checked along with type of specimen received, clinical and radiological details mentioned in the requisition form. Photograph the specimen from both aspects for records purpose.
- The type of growth in the specimen is noted (Ulcerative/ infiltrative etc.)
- Insert a cotton wick soaked in formalin into the lumen of the intact segment containing tumour and fix the entire specimen in appropriate volume of formalin overnight or for 48 hours.
- Cut serial slices of not more than 5 mm thick of the segment containing tumour (which is kept intact and inked appropriately) in a transverse manner starting from 1 cm above and ending 1cm below the limits of the tumour.
- Examine the rest of the bowel segment for any abnormality like synchronous carcinoma, features of inflammatory bowel disease, polyp s etc.

- Lymph nodes are not dissected off the specimen 'before' tumour sampling to avoid disruption of serosa and non-peritonealised surface. Lymph nodes present in the sections taken for tumour are included in situ without dissection.
- As per CAP guidelines, a minimum of 12 lymph nodes should be examined to avoid tumour under-staging.

Fig 3. Shows gross specimen of Right hemicolectomy specimen which is distended and on cut surface shows a proliferative growth obstructing the lumen

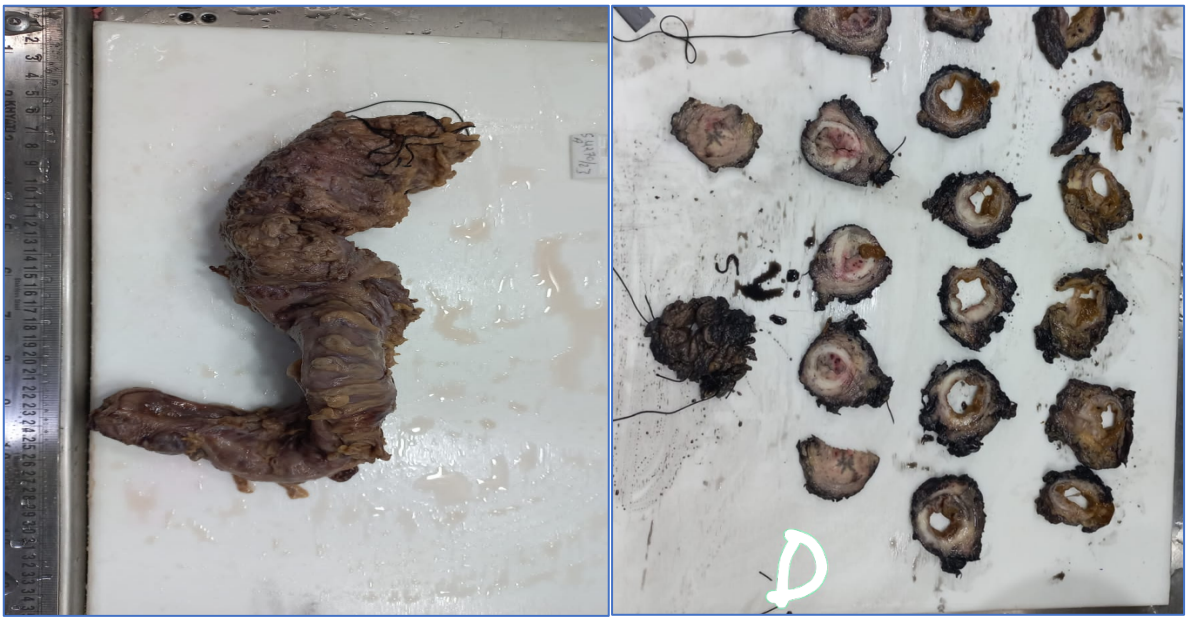

Fig 4. Shows Abdominoperineal resection specimen where the non-peritonealised surface is inked and serial sections of the tumor are taken

Staining Protocols:

Hematoxylin and Eosin

1) Deparaffinize sections in xylene

2) Hydrate the tissue via graded alcohol in decreasing order of strength 100%, 90%, 75%

3) Staining with hematoxylinto see the nucleus for 3-5 min

4) Wash in running tap water for bluing

5) Differentiate in 1% acid alcohol

6) Wash in running tap water

7) Counterstain with Eosin Y for 10 min

8) Wash under running tap water for 1-5 min

9) Clear with 2 changes of xylene for 2 minutes

10) Mount in DPX

- After the interpretation of histopathological features, IHC was performed on selected blocks of each case.

Automated IHC was performed on representative blocks of paraffin-embedded tissue following the below mentioned protocol:

Figure 5: The automated immuno-stainer (VENTANA BenchMarkXT) used to immunostain the slides using the Optiview kit in the department of pathology and lab medicine, AIIMS Bhopal

To perform IHC using anti BRAFV600E antibody on all the cases, an automated immunostainer (VENTANA BenchMarkXT) was used. This machine can stain 30 slides in a single run.

Positive control: For KRAS**,** the tissue for positive control used was the small intestine where the cytoplasm of crypts stain positive. The clone used was KRAS Recombinant Mouse Monoclonal Antibody from Invitrogen(Mouse monoclonal clone 9.13).

For BRAF, the tissue for positive control used was testis where the cytoplasm of the Seminiferous tubules stain positive.

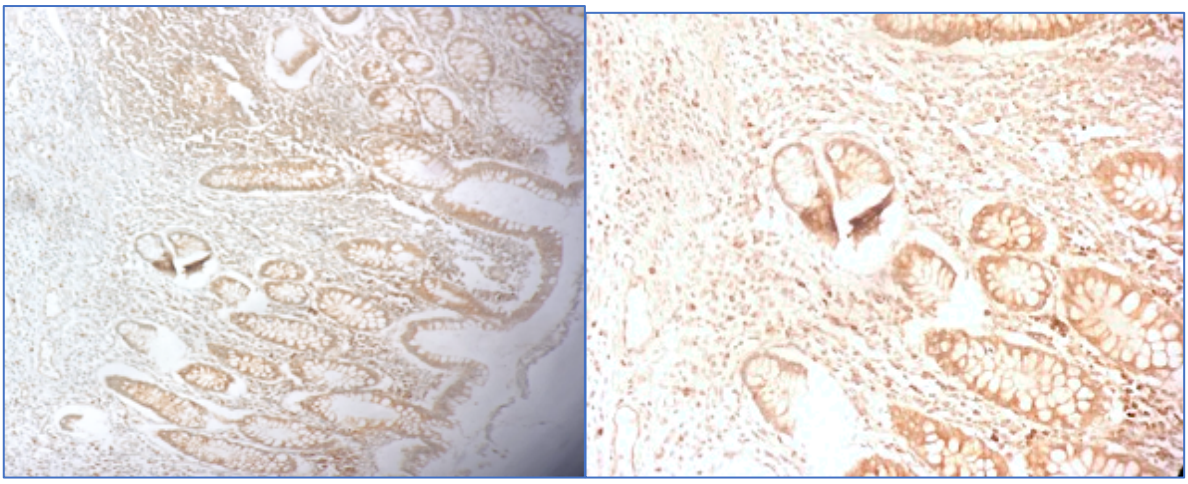

Fig 6: Showing normal crypts of intestine staining positive for KRAS taken as positive control (10X and 20X respectively)

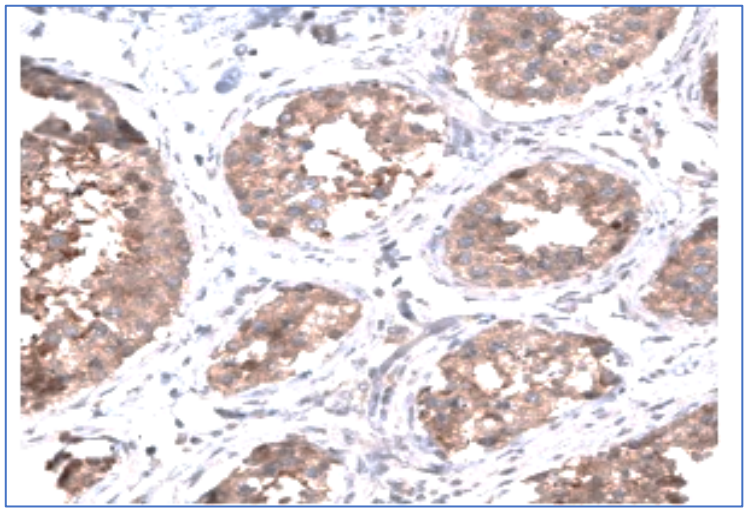

Fig 5: Showing Seminiferous tubules of testis staining positive for BRAF taken as positive control (20X)

Negative control: The staining of the blood vessel wall, inflammatory cells and or any nuclear staining was considered and staining comparable to these tissues was taken as negative or weak staining.

IHC was performed following the protocol mentioned below using Ultraview secondary antibody kit:

1. Machine preparation:

a) Product Registration: Product registration was done using a registration probe attached to the computer. This probe was need to be touched twice to the metal memory button on the reagent pack, which reads and transfers the product information to the computer.

b) Printing barcode labels: Barcodes were printed for the pre-set protocol for this antibody. For this first went to the 'Home' page and then pressed the 'Create Label' button followed in order by the 'Protocols button' then the 'Slide Labels' option and then 'label template 'and protocols.

c) Pre-Run Preparation: All the reagent bottles were checked for reagent availability and if empty were refilled after product registration as mentioned above. The waste module was emptied if full; the pH of the Reaction Buffer, EZ prep and other buffers are checked.

d) Starting a Run: Slide pads were washed by selecting the washing option and a reagent tray was installed on the top of the staining machine in reagent slots. Printed labels were pasted to the top left corner of the slide.

e) Loading Slides: At the Instrument View, the slide drawer is opened and slides were positioned over the slide pads in a way that labels facing up and inward. It was made sure that slides were seated well over the slide pad. Now the number of slides put in drawer is counted and entered into the system.

f) Now at the Instrument View, the running mode button was pressed. The system now reads reagent and slide bar codes then calculates the time after which primary antibody will be applied and starts the run.

g) We have used the KRAS Recombinant Mouse Monoclonal Antibody from Invitrogen(Mouse monoclonal clone 9.13) detection of mutated KRAS and BRAF Rabbit Monoclonal Antibody (Rabbit Monoclonal, clone RM8) from Invitrogen; USA for the detection of mutant BRAF V600E mutation.

h) Control**:** For KRAS**,** the tissue for positive control used was the small intestine where crypts stain positive. The clone used was KRAS Recombinant Mouse Monoclonal Antibody from Invitrogen; USA (Mouse monoclonal clone 9.13).

And for BRAF, the tissue for positive control used was testis where the Seminiferous tubules stain positive.

S. No	**Antibody**	**Manufacturer**	**Dilution**
1.	**KRAS**	**Invitrogen**	**1:100**
2.	**BRAF**	**Invitrogen**	**1:100**

Preparation of slides and automatic IHC protocol used for IHC:

The protocol for both KRAS and BRAF is similar.

a) Firstly, the slides were put on the hot plate for 55 minutes to fix the tissue on the slides and deparaffinise it. Now slides were kept in milk for 5 minutes to prevent unspecific binding to the hydrophobic side chains of proteins present in the tissue.

b) Antigen was retrieved using heat induced epitope retrieval method for that cell conditioning with CC1 is done for 90 minutes.

c) Quenching of the endogenous pre- primary peroxidase is done followed by incubation with primary antibody for 1 hour.

d) Now slides were incubated with one drop of ultraview HQ universal linker and one drop of ultraview HRP multiplier for twelve minutes each.

e) Then slides were incubated with 1 drop of haematoxylin and one drop of bluing reagent for 12 minutes and 4 minutes respectively.

f) After completion of the run primary antibody is applied over the slides and the start button of the machine is pressed.

g) Unload Completed Slides: When the run is completed, the drawer is opened and stained slides were removed.

h) After Run: Now slides were rinsed in warm soapy water so that any remaining

reagents were removed. Now slides were dried and a coverslip was mounted using DPX and examined.

Outcome Measure and Interpretation of IHC:

The cytoplasmic staining pattern for both KRAS and BRAF have been recorded for percentage of cell positivity, the intensity of stain and cytoplasmic localization and grading was be done. For this purpose the tables, given below have been used.

IHC for BRAF will be considered IHC positive if 2+ or 3+ staining intensity will be found in at least 10% of the cells.

Table 3: IHC interpretation protocol for KRAS in colorectal adenocarcinoma (71)		
Staining grade	Intensity	Percentage of cells
0	Absent	0-10 % of cells
1	Weak	11-30%
2	Moderate	31-50%
3	Intense	>50%

Table 4: BRAF IHC interpretation protocol for colorectal carcinoma(102)			
Staining grading intensity	Percentage of Stained cells	Staining Pattern	Interpretation of result
0	0-10%	Absence of staining	Negative
1+	10-33%	Weak cytoplasmic staining	Negative
2+	33-66%	Moderate cytoplasmic staining	Positive
3+	>66%	Intense cytoplasmic staining	Positive

5. RESULTS

During this study conducted at the Department of Pathology and Lab Medicine in collaboration with the Department of General Surgery and Department of Surgical Oncology, a total of 50 diagnosed Colorectal adenocarcinoma cases were included during the period of January 2018 to June 2023.

The results of interpreting various histomorphological features and KRAS and BRAF IHC expression colorectal adenocarcinoma in the present study are as follows:

1. Type of specimens

Table 5. Summary of different types of specimens received	
Type of Specimen	N= 50
1. Surgical Resections	36 (72.00%)
2. Biopsy	14 (28.00%)

During the period between January 2018 to June 2023 we received 36 surgical resection cases and 14 biopsies and were diagnosed as Colorectal Adenocarcinoma. More number of surgical resection cases were received.

2. Types of Specimens based on sex of patients

Table 6. Summary of different types of specimens received based on sex of the patients			
Sex	Overall, N = 50	Biopsy, N = 14	Surgical resection, N = 36
Female	25 (50.00%)	9 (64.29%)	16 (44.44%)
Male	25 (50.00%)	5 (35.71%)	20 (55.56%)

Out of a total 50 cases overall Male-to-female ratio was 1:1 (Including Surgical resection and biopsy cases). The female patients were comparatively more who underwent biopsy procedures for colorectal adenocarcinoma. In contrast, male patients were more who underwent surgical resections.

3. Type of specimen and Age of patient

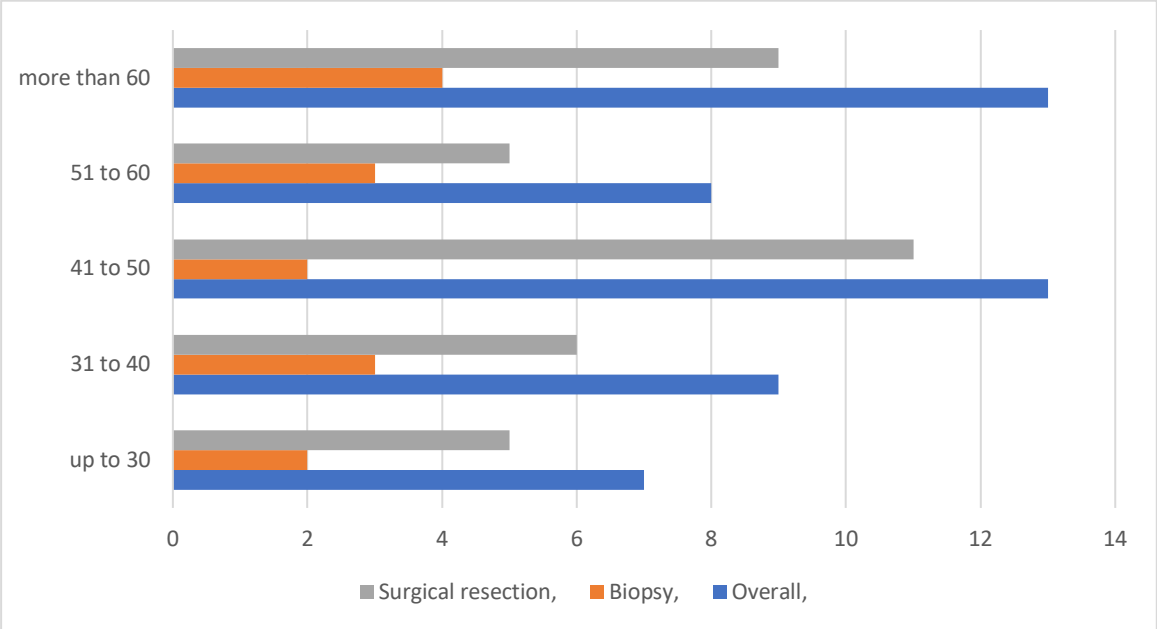

Fig 8. The bar graph shows age wise categorisation of biopsy and surgical resection specimens

Out of total 50 cases, maximum cases received were in the age group of 41-50 years (11/50 cases). The maximum number of resection specimens received were also in the same age group. Maximum number of biopsies were received in the age group > 60 years.

The age of the youngest patient was 17 yrs and the oldest patient was 72 yrs. The mean age of patients including resection and biopsy cases was 48.26 years.

4. Site of tumor

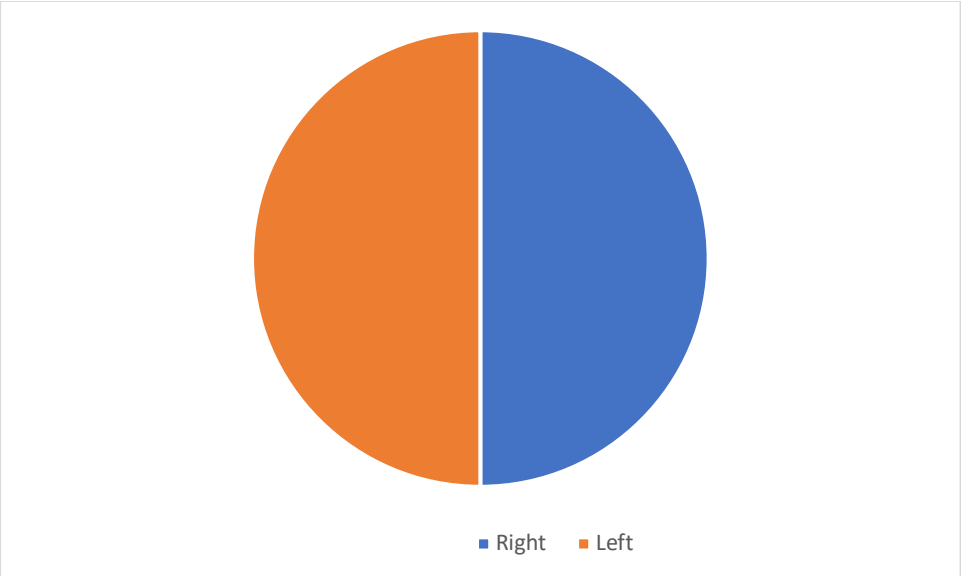

Fig 9. The above pie chart describes the distribution of the site of tumor

Among the 50 cases, the site of tumor when broadly divided into Right and Left, it was seen that the ratio is 1:1.

Table 7. Summary of various sites of colorectal adenocarcinoma (Including biopsy and resection cases) ranging from ileocaecal junction to anal verge.	
Site of tumor	N = 50
Caecum	13 (26.00%)
Ascending Colon	10 (20.00%)
Rectum	10 (20.00%)
Recto-Sigmoid Region	7 (14.00%)
Sigmoid Colon	5 (10.00%)
Descending colon	2 (4.00%)
Ileo-caecal Junction	2 (4.00%)
Anal Verge	1 (2.00%)

When the site of tumor is divided based on specific site in the intestine, of the total 50 cases in the present study, the most common site of Colorectal adenocarcinoma was Caecum. Followed by Ascending colon and rectum. The least common is Anal verge where only one case was reported.

5. Histologic Type

Table 8. Categorisation of the histological types seen in surgical resection and biopsy cases in total	
Histologic Type	N= 50
Adenocarcinoma	41 (82.00%)
Mucinous adenocarcinoma	7 (14.00%)
Mixed neuroendocrine-non-neuroendocrine neoplasm with Adenocarcinoma component	1 (2.00%)
Signet-ring cell carcinoma	1 (2.00%)

When the histological type was classified for all 50 cases, most common type was Adenocarcinoma (n= 41, 82%), whereas least common were Signet ring cell Carcinoma (Signet ring cells > 5-%) (n=1, 2%) and Mixed neuroendocrine-non-neuroendocrine neoplasm with Adenocarcinoma component (n=1, 2%). Mucinous adenocarcinoma (Mucin component is > 50%) were seen in 7 cases.

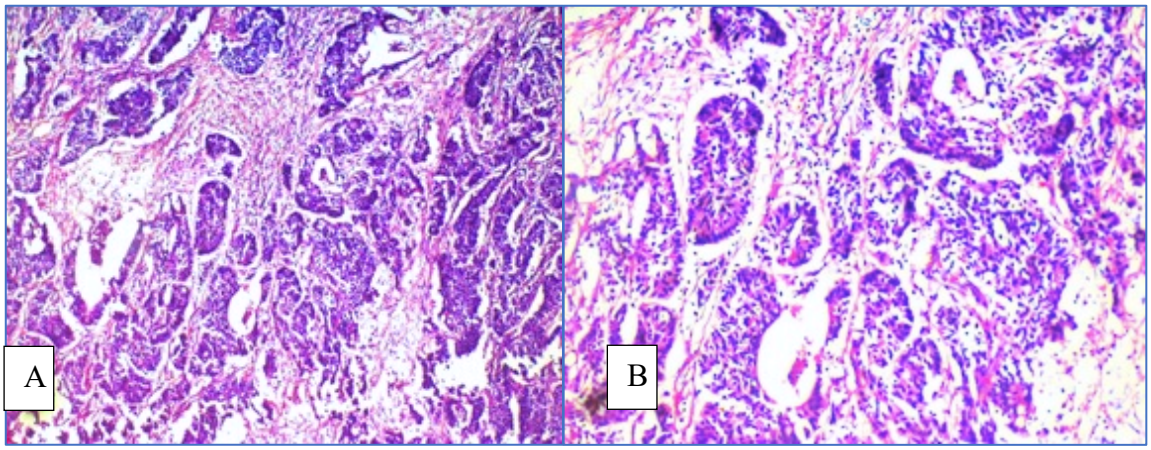

Fig 10. Shows Adenocarcinoma Colon where the tumor is arranged in Glandular architecture (A and B; H and E stain 10X and 20X respectively).

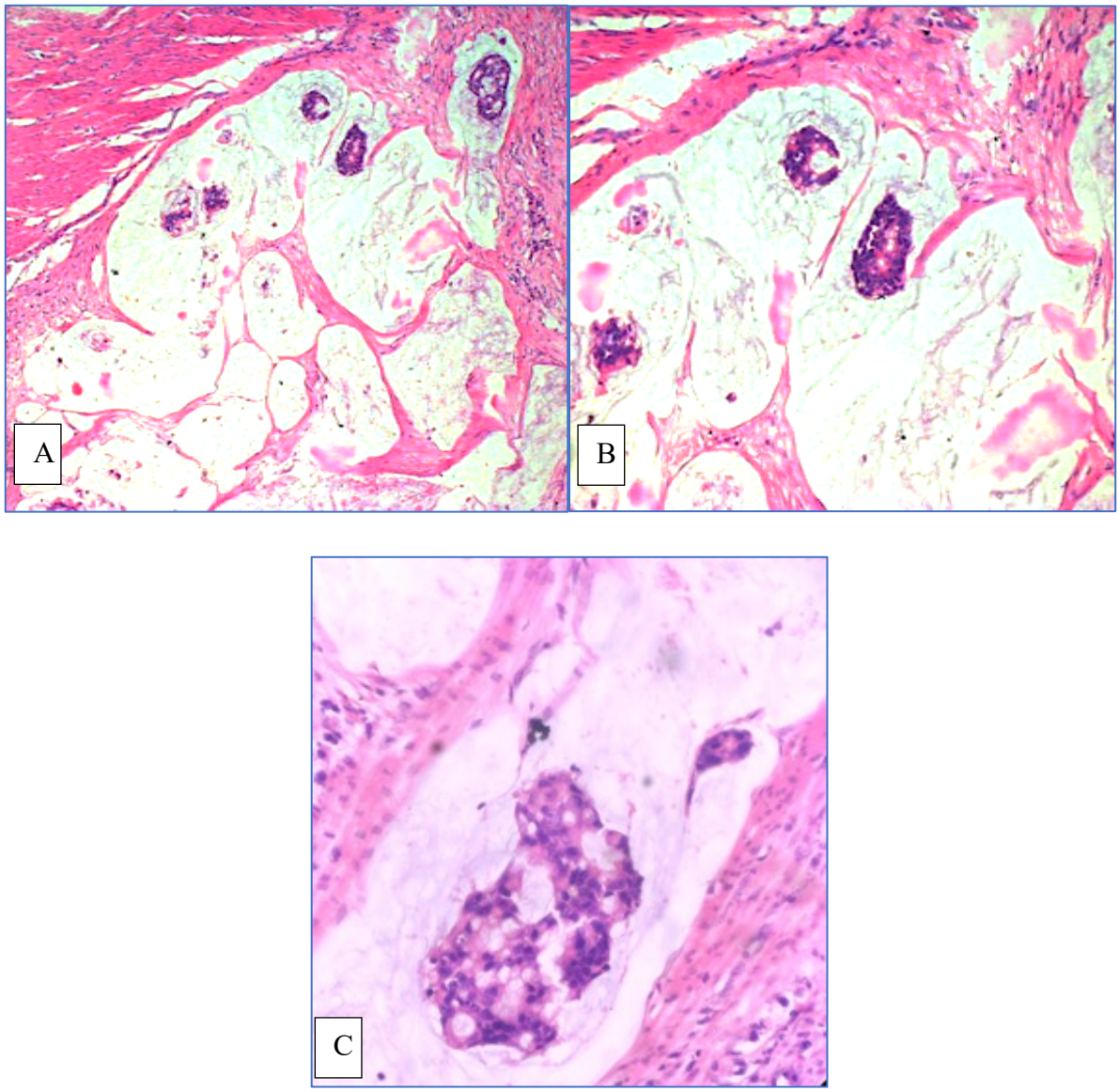

Fig 11. Shows Mucinous adenocarcinoma where the mucinous component is > 50% and also the tumor cells are seen floating the extracellular mucin pools (A, B and C; H and E stain at10X, 20X and 40X respectively)

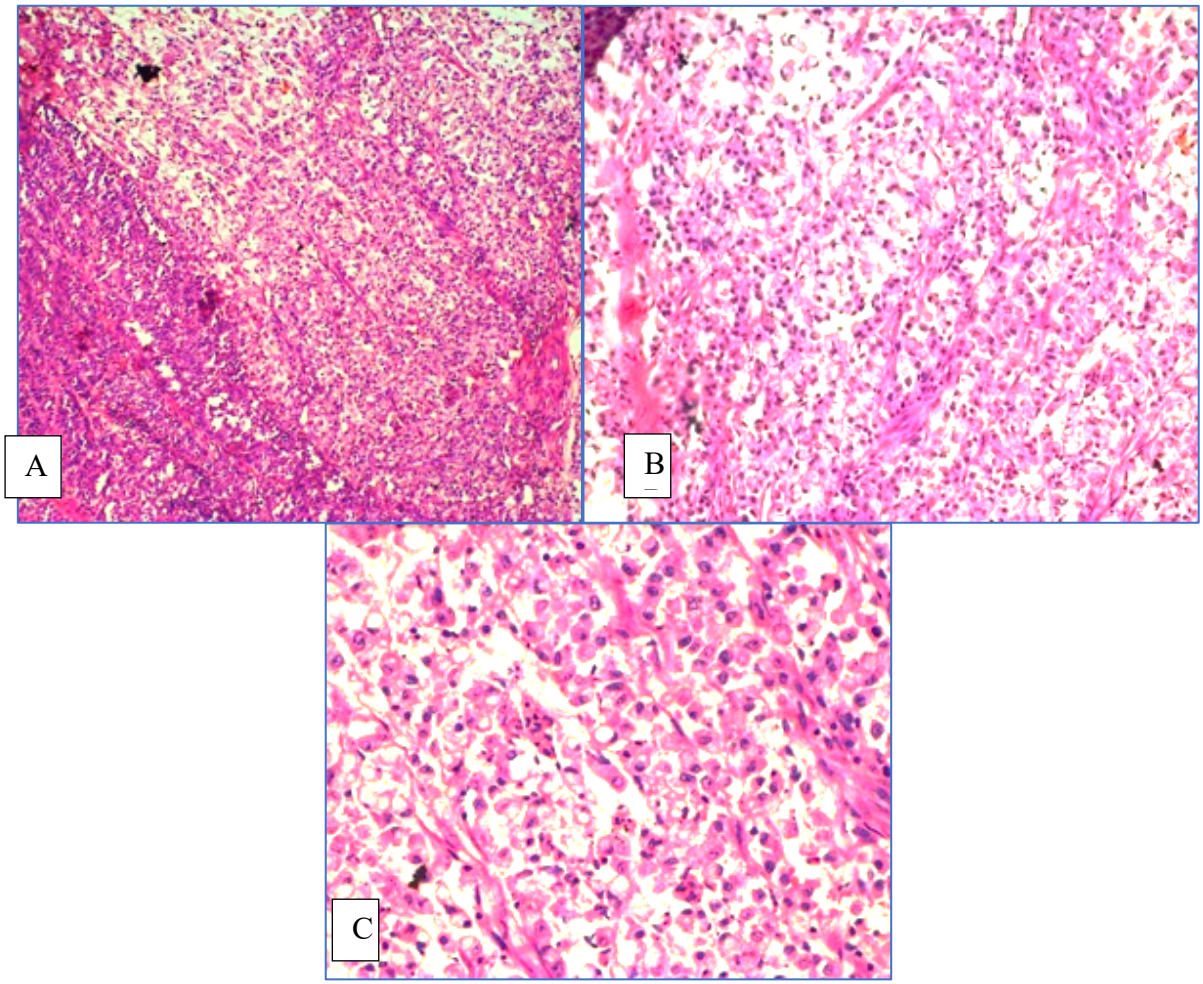

Fig 12. Shows Signet cell Carcinoma. A) H and E stain at 10 X shows sheets of tumor cells. B) H and E stain at 20 X shows few clusters of tumor cells C) At 40X we see the morphology of the tumor cells with intracellular mucin pushing the nucleus to one side, flattened against the cytoplasmic membrane, and creating a signet ring appearance.

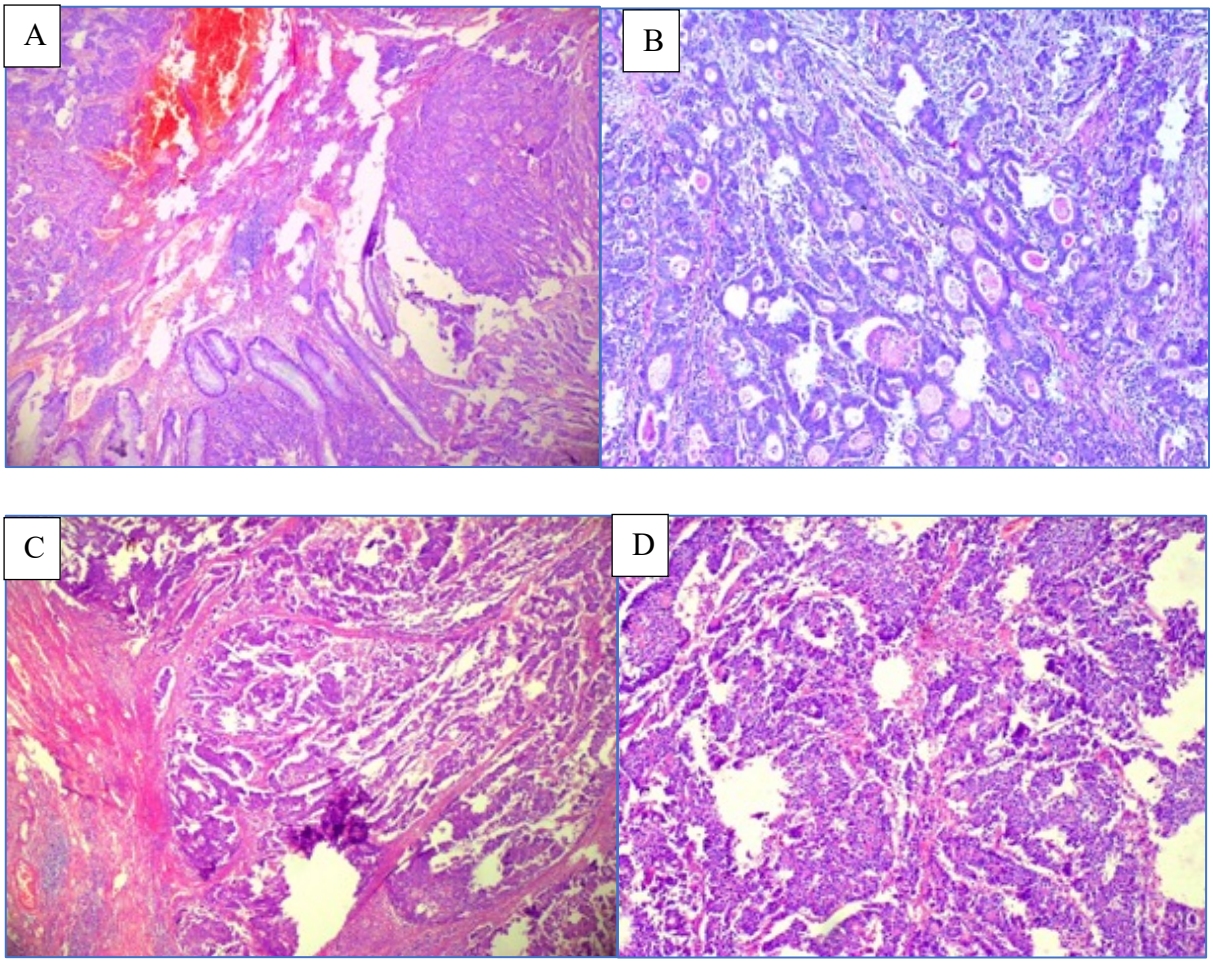

Fig 13. Shows Mixed neuroendocrine and non-neuroendocrine tumor with adenocarcinoma component. A) and B) Adenocarcinoma component of the tumor with glandular architecture (H and E stain 4X and 10X respectively). C) and D) Neuroendocrine component of the tumor arranged in cords and sheets (H and E stain 4X and 10X respectively).

6. Histological Grade

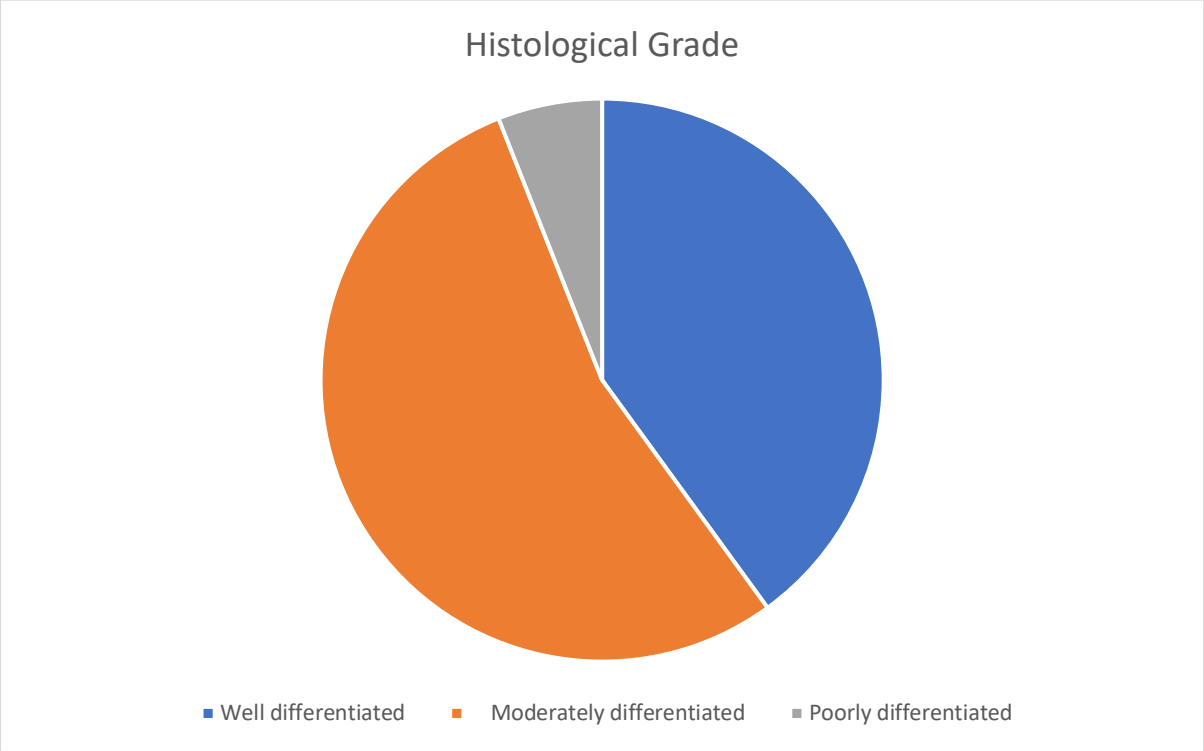

Fig 14. Pie chart showing the number of cases received and their histological grade categorised in Well, Moderate and Poorly Differentiated.

Based on the categorisation of the cases for histological types including surgical resection and biopsy samples, most common cases were moderately differentiated (Gland formation 50-95%) followed by well-differentiated (Gland formation > 95%) and poorly differentiated (< 50%). One case which was reported as Mixed neuroendocrine-non-neuroendocrine neoplasm with Adenocarcinoma component has been placed under poorly differentiated due to morphology in the adenocarcinoma component.

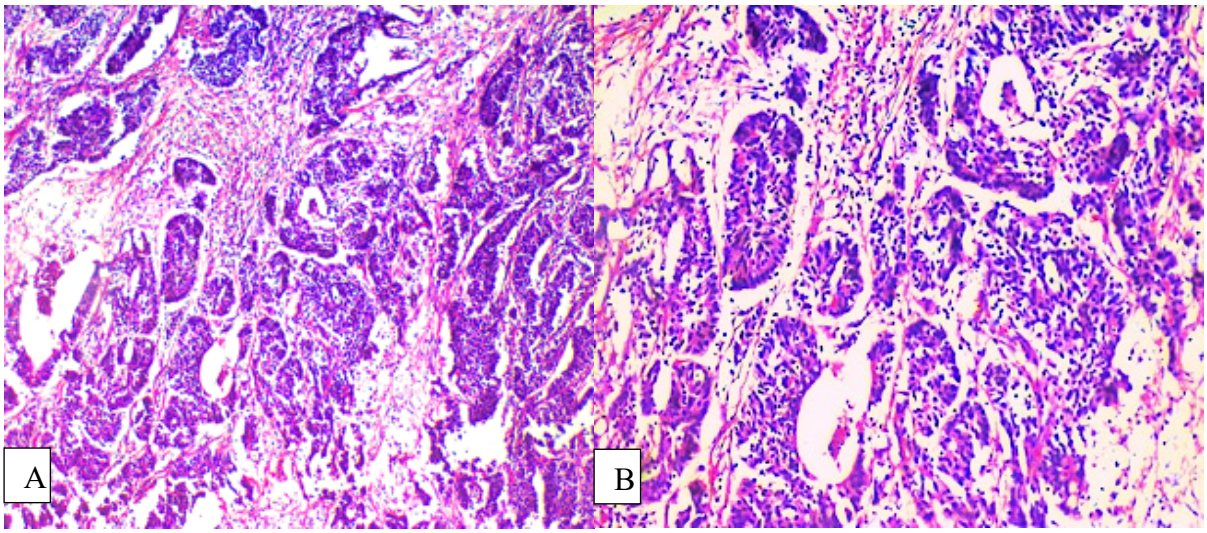

Fig 15. Shows Well-differentiated Adenocarcinoma. A) and B) H and E stain at 10 X and 20X respectively show tumor arranged predominantly (> 95%) in glandular pattern.

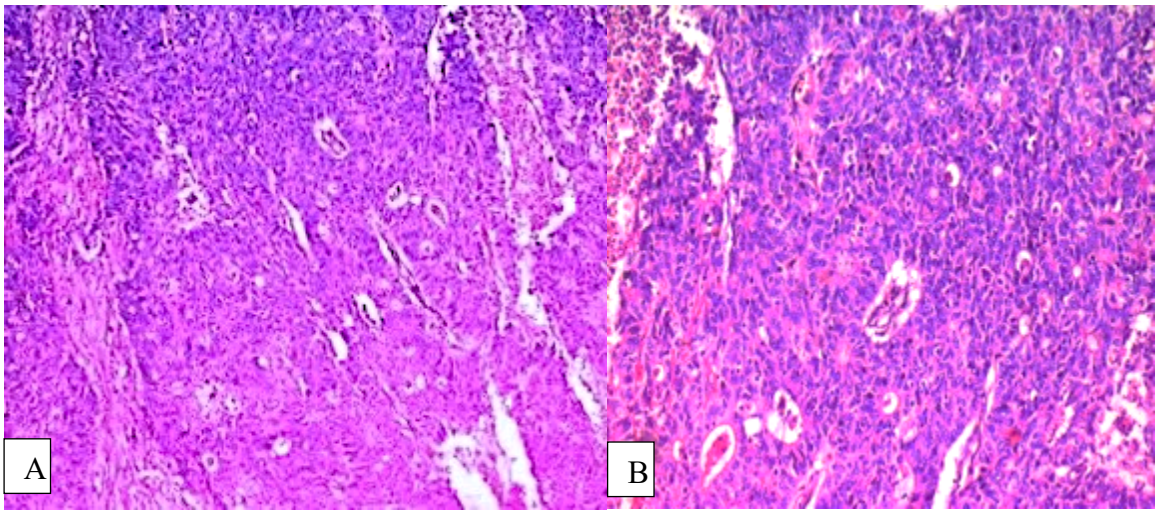

Fig 16. Shows Moderately-differentiated Adenocarcinoma. A) and B) H and E stain at 10 X and 20X respectively show tumor arranged in glandular pattern (50-95%) and also in sheets and trabeculae.

7. Tumor Extent

Table 9. The table shows the extent to tumor invasion	
Tumour Extent	N= 36
Invades submucosa	0 (0%)
Invades into muscularis propria	10 (26.80%)
Invades through muscularis propria into the pericolorectal tissue	16 (45.95%)
Reaches up to serosa	8 (21.92%)
Invades beyond serosa	2 (5.41%)

The extent of tumor was assessed on the H and E slides of surgical resection cases. Most of the tumor had an extent upto the pericolorectal tissue (16/36; 45.95%).

8. Tumor size: The average tumor size in the surgical resection(N=36) cases was 5.6 cm.

9. Association between tumor size and lymph node staging

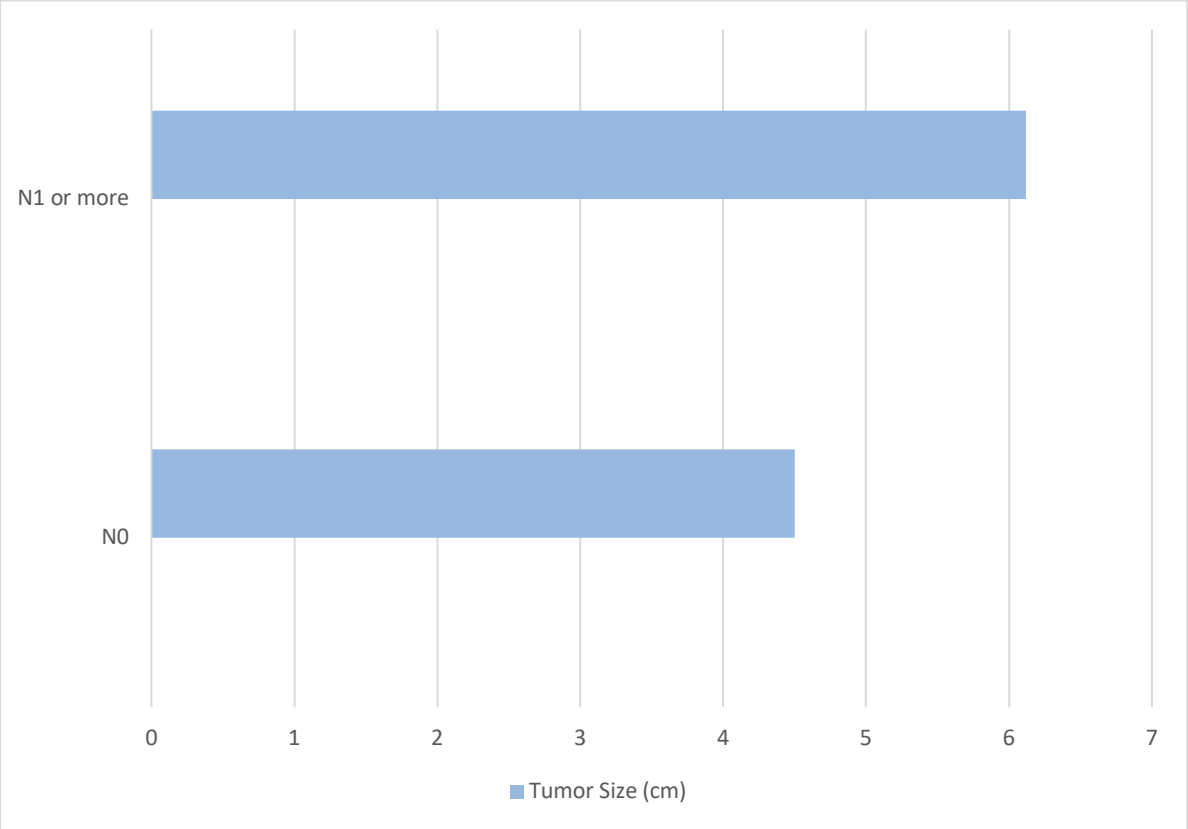

Fig 17. Comparison between tumor size and Lymph node staging.

When the tumor size was compared to the lymph node staging, the positive lymph node cases had larger tumor size compared to the cases having negative lymph node cases. Statistically no significant data was obtained with p-value 0.076. Nine cases had no nodal metastasis and showed an average tumor size of 4.5 cm. Whereas the cases which had positive lymph metastasis or any seb serosal or mesenteric deposits (N1c) had tumor size of 6.12cm.

10. Tumor budding

Table 10. Average number of tumor buds	
Number of Tumor Buds	3.72 (3.8)

The number of tumor buds was evaluated based on the number of tumor buds in the intratumor and peritumor site of the tissue in the surgical resection cases (N=36). Tumor buds were counted in number and then classified based on a 3 tier system of low, intermediate and High

The average number of tumor buds found was 3.72.

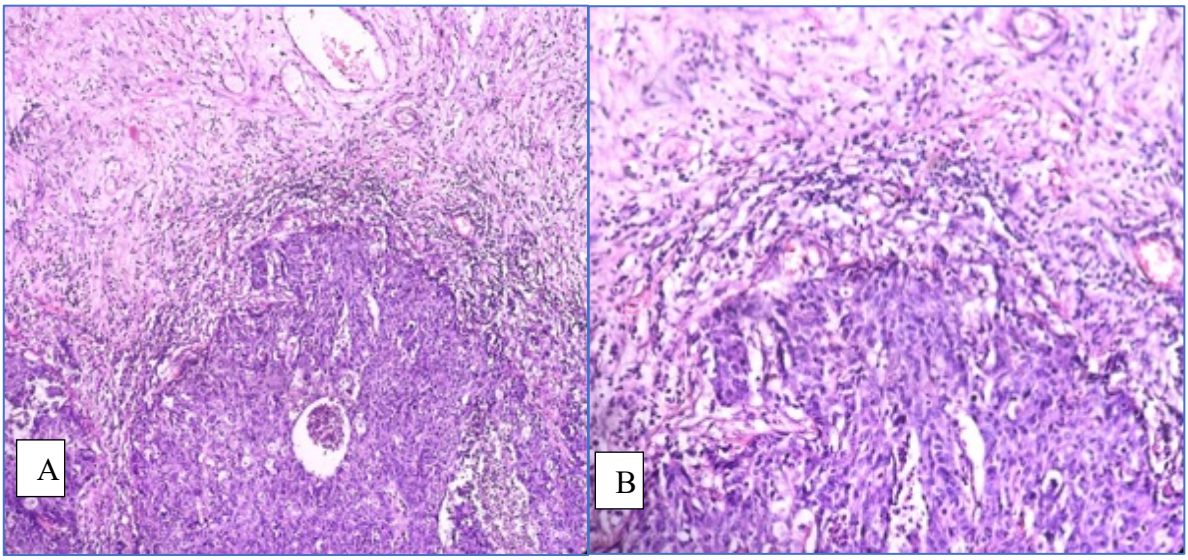

Fig 18. Shows Low tumor bud (0-4) A) and B)The images show no tumor buds at the advancing end of the tumor (H and E 10X and 20X respectively).

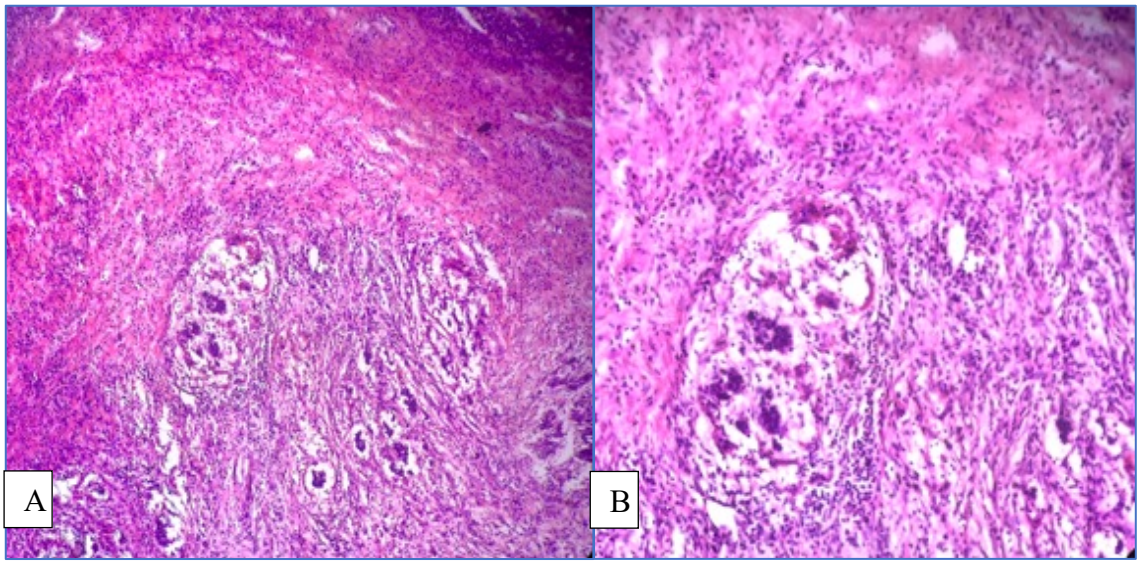

Fig 19. Shows Intermediate tumor buds(5-9). A) and B)The image shows few tumor buds at the advancing end of the tumor (H and E 10X and 20X respectively).

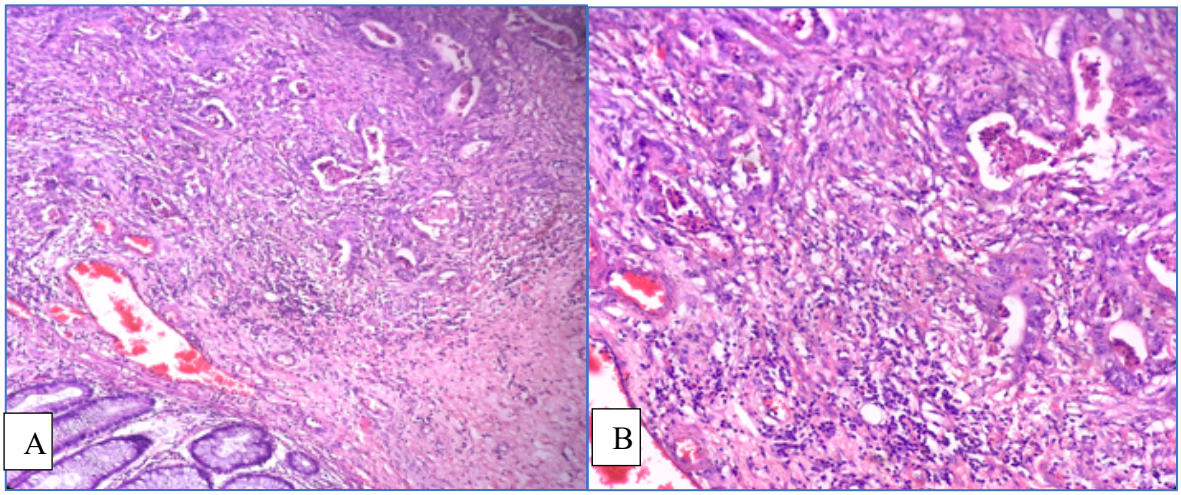

Fig 20. Shows High (>10 tumor buds) A) and B)The image shows no tumor buds at the advancing end of the tumor (H and E 10X and 20Xrespectively).

11. Lymphovascular invasion

Table 11. Number of cases with lymphovascular invasion	
Lymphovascular Invasion	
Absent	40 (80.00%)
Present	10 (20.00%)

Most of the surgical resection cases and biopsy cases had no lymphovascular invasion. Only 10/50 cases had evidence of lymphovascular invasion.

12. Association between lymph node staging and tumor bud score

Table 12. Lymph node status (Positive and Negative) and Tumor bud score			
	Negative lymph node (N0)	Positive lymph nodes (N1 and above)	Total
Tumor Bud Score			
Low	22 (61.11%)	0 (0.00%)	22 (61.11%)
Intermediate	3 (8.33%)	4 (11.12%)	7 (19.44%)
High	2 (5.56%)	5 (13.90%)	7 (19.44%)
Total	27 (75.00%)	9 (25.00%)	36 (100.00%)
[1] Fisher's exact test			

Out of 36 resection cases, All 22 cases with no lymph node metastasis had low tumor bud score. 3/7 cases with negative lymph node status had intermediate score and 4/ 7 had positive lymph node status. 5/7 cases high tumor bud score which also had positive lymph node status.

Rest 2 cases had negative lymph nodes. A statistically significant association was found between lymphnode staging and tumor bud score. (p= <0.001).

13. Positive KRAS immunohistochemical expression

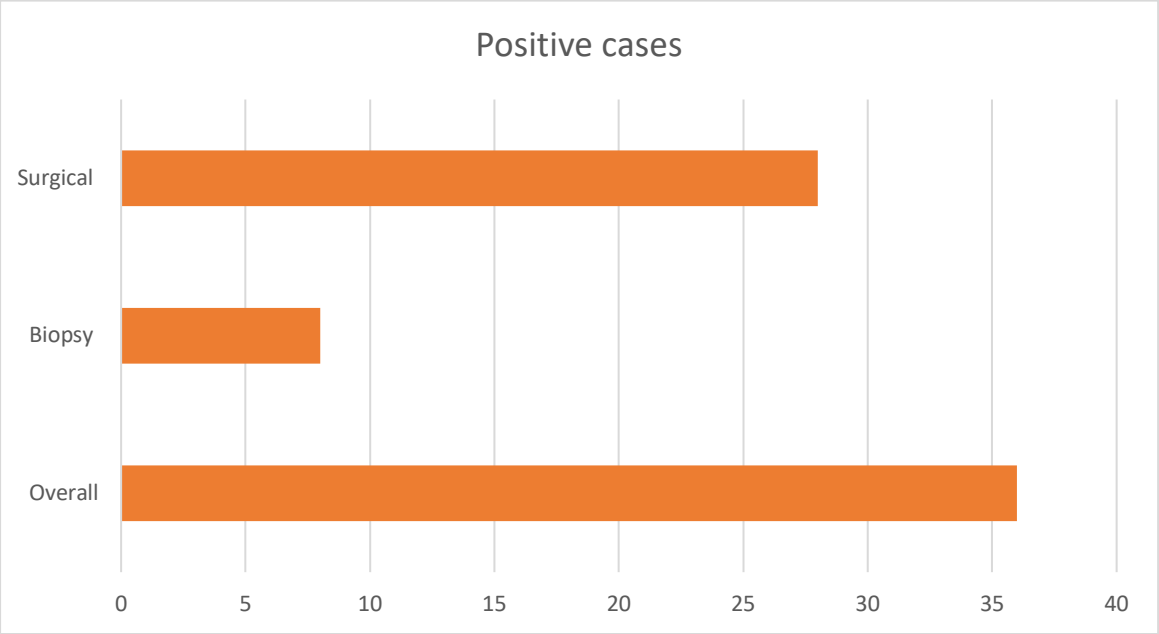

Fig 21. Total number of cases which were positive for KRAS (including biopsy and resection cases)

Out of a total 50 cases, KRAS positive expression was seen in 36 cases. 08 biopsy cases and 28 surgical resection cases were positive.

14. KRAS Immunohistochemical expression with Grade

Table 13. Grading of KRAS expression in biopsy and surgical resection cases			
	Overall, N = 50[1]	Biopsy, N = 14[1]	Surgical resection, N = 36[1]
Grade-KRAS			
0	3 (6.00%)	1 (7.14%)	2 (5.56%)
1	11 (22.00%)	5 (35.71%)	6 (16.67%)
2	13 (26.00%)	0 (0.00%)	13 (36.11%)
3	23 (46.00%)	8 (57.14%)	15 (41.67%)
[1] Fisher's exact test			

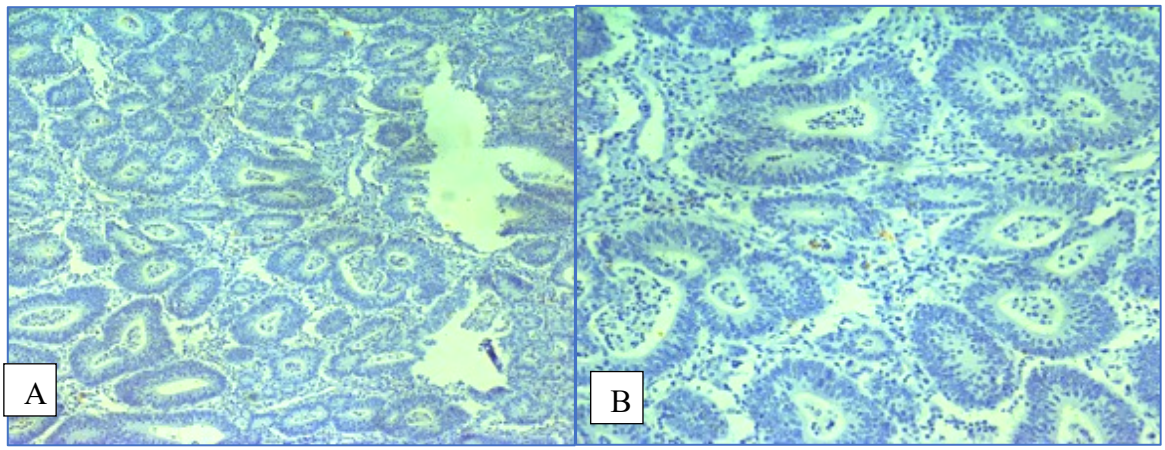

Fig 22. Shows KRAS immunostaining (Grade 0) where no cells show staining; A) & B); 10X & 20X respectively

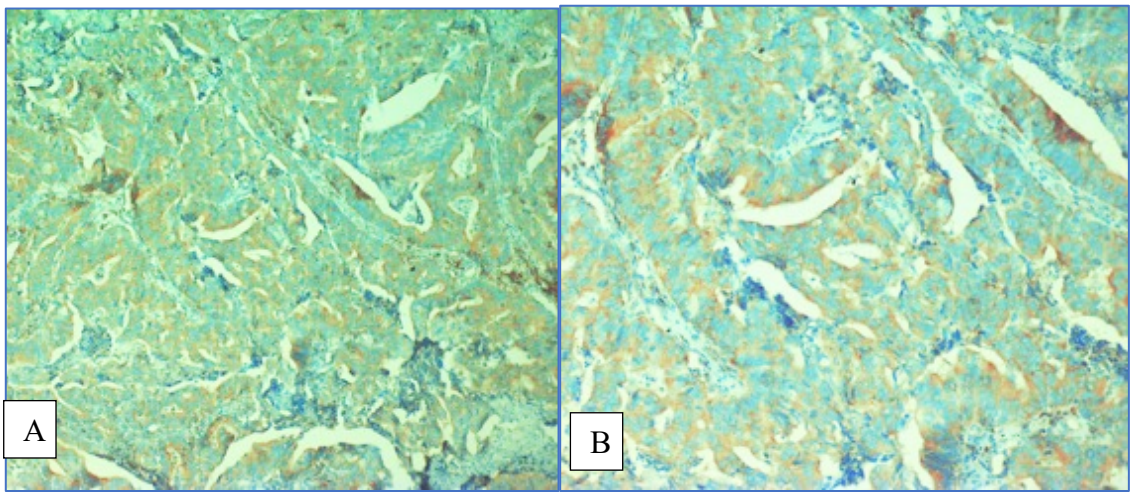

Fig 23. Shows KRAS immunostaining (Grade 1) where 11-30% cells show weak intensity staining; A) 10X & B) 20X respectively

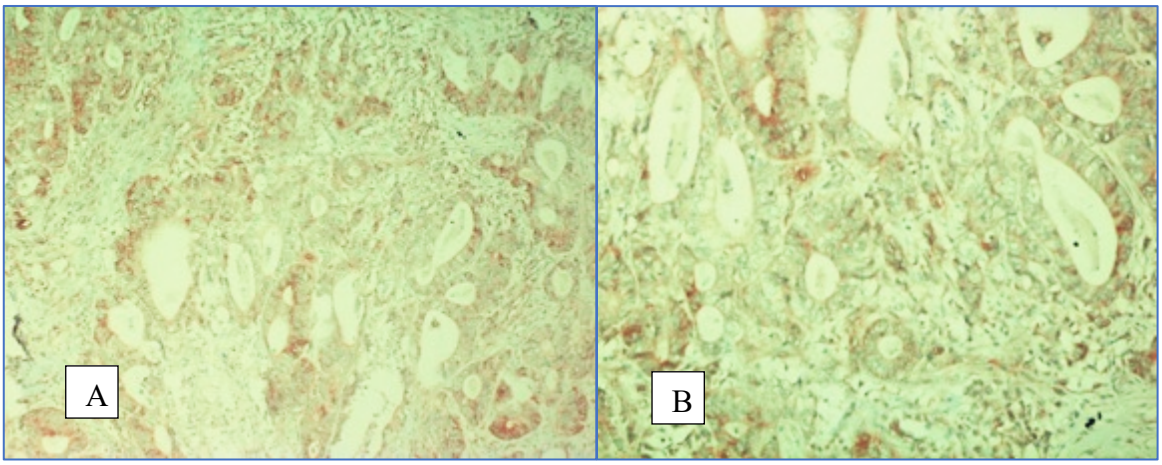

Fig 24. Shows KRAS immunostaining (Grade 2) where 31-50% cells show moderate intensity staining; A) 10X & B) 20X respectively

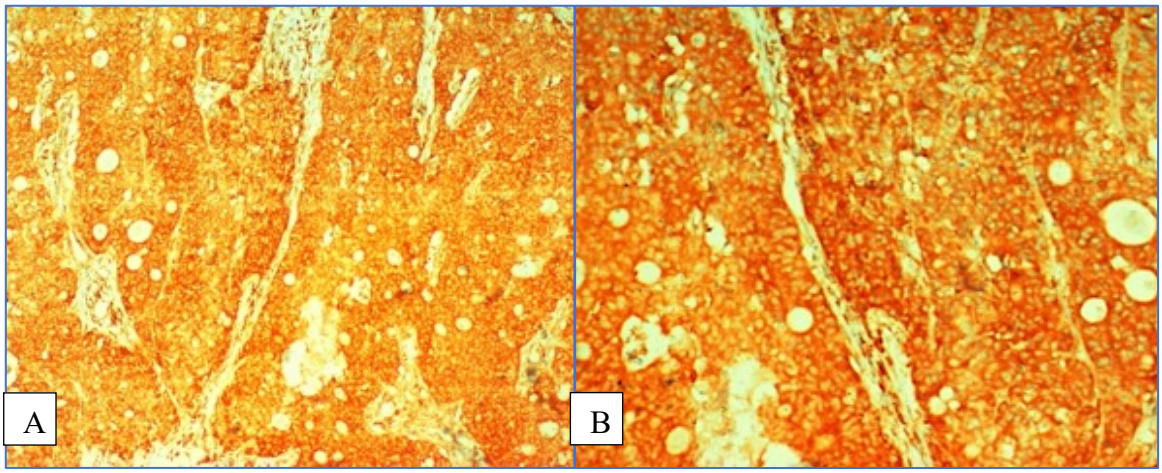

Fig 25. Shows KRAS immunostaining (Grade 3) where >50% cells show strong intensity staining; A) 10X & B) 20X respectively

Out of a total 36 resection cases, 5 cases were mucinous adenocarcinoma. All five cases are KRAS positive**.** 1 Signet ring cell carcinoma was also KRAS positive.

15. KRAS immunohistochemical expression and Histological grade

Table 14. The table summarises the number of cases based on histological grade and Grade of KRAS expression

	Grade-KRAS				
	0	1	2	3	Total
Histological Grade					
Well differentiated	0 (0.00%)	4 (20.00%)	3 (15.00%)	13 (65.00%)	20 (100.00%)
Moderately differentiated	2 (8.00%)	7 (28.00%)	10 (32.00%)	8 (32.00%)	27 (100.00%)
Poorly differentiated	1 (33.33%)	0 (0.00%)	0 (0.00%)	2 (66.67%)	3 (100.00%)
Total	3 (6.00%)	11 (22.00%)	13 (26.00%)	23 (46.00%)	50 (100.00%)

[1]Fisher's exact test

On comparing the grade of KRAS expression and histological grade in both surgical resection and biopsy cases, no statistically significant association was found between histological type and KRAS positivity (p= 0.58).

16. Association between KRAS immunohistochemical expression and Lymphovascular invasion

Table 15. KRAS expression and Lymphovascular invasion		
KRAS immunohistochemical expression		
	Positive	Negative
Lymphovascular Invasion		
Present	10 (100.00%)	0 (0.00%)
Absent	26 (65.00%)	14 (35.00%)
Total	36 (72.00%)	14 (28.00%)
[1]Fisher's exact test		

On comparing the KRAS expression in two broad categories i.e. positive and negative expression with lymphovascular invasion, 10/ 36 cases with positive KRAS expression showed lymphovascular expression. 26/36 cases with positive KRAS expression did not have lymphovascular invasion. No cases with negative KRAS expression had lymphovascular invasion.14 cases with negative KRAS expression did not have any evidence of lymphovascular invasion. A statistically significant association was found between KRAS expression and lymphovascular invasion (p= 0.045).

17. Association between KRAS immunohistochemical expression and Tumor bud score

Table 16. KRAS expression and Tumor bud score			
	Positive	Negative	Total
Tumor Bud Score			
Low	26 (76.47%)	8 (23.53%)	34 (100.00%)
Intermediate	4 (44.44%)	5 (55.56%)	9 (100.00%)
High	6 (85.71%)	1 (14.29%)	7 (100.00%)
Total	36 (72.00%)	14 (28.00%)	50 (100.00%)
[1]Fisher's exact test			

Out of a total 50 cases, 34 cases had low tumor bud scores with 26/34 cases showing positive KRAS expression. 9 cases had intermediate tumor bud score and 4/9 cases had positive KRAS expression. 7 cases had high tumor bud score and 6/7 cases had positive KRAS expression. Statistically, no significant association was found between KRAS expression and Tumor bud score ($p= 0.14$).

18. Grading of KRAS immunohistochemical and association with Tumor bud score

Table 17. Association between KRAS grade and Tumor bud score					
	Grade-KRAS				
	0	1	2	3	Total
Tumor Bud Score					
Low	1 (2.94%)	7 (20.59%)	7 (20.59%)	19 (55.88%)	34 (100.00%)
Intermediate	2 (22.22%)	3 (33.33%)	2 (22.22%)	2 (22.22%)	9 (100.00%)
High	0 (0.00%)	1 (14.29%)	4 (57.14%)	2 (28.57%)	7 (100.00%)
Total	3 (6.00%)	11 (22.00%)	13 (26.00%)	23 (46.00%)	50 (100.00%)
[1]Fisher's exact test					

On comparing the grade of KRAS immunohistochemical expression and tumor bud score, 2/7 cases had grade 3 expression and high tumor bud score. No sigificant association was found with $p = 0.11$.

19. Grading of KRAS immunohistochemical and association with T staging

Table 18. Association between KRAS grade and T staging

	0	1	2	3	Total
T staging					
1	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
2	1 (8.33%)	3 (25.00%)	3 (25.00%)	5 (41.67%)	12 (100.00%)
3	1 (4.55%)	2 (9.09%)	9 (40.91%)	10 (45.45%)	22 (100.00%)
4	0 (0.00%)	1 (50.00%)	1 (50.00%)	0 (0.00%)	2 (100.00%)
Total	2 (5.56%)	6 (16.67%)	13 (36.11%)	15 (41.67%)	36 (100.00%)

[1]Fisher's exact test

For the surgical resection cases(n=36) T staging and Grade of KRAS immunochemical expression association was done. No T1 stage tumors were seen in our study. In T2 stage, 8/12 cases showed positive KRAS expression(Grade 2+ Grade 3). In T3 stage, 19/22 cases had positive KRAS expression (Grade 2+ Grade 3). For T4 stage tumors, 1/2 case had positive KRAS expression(Grade 2+ Grade 3). Statistically, no significant association was found between KRAS expression and Tumor bud score (p= 0.5).

20. KRAS immunohistochemical expression and Node staging

Table 19. Association between KRAS immunohistochemical expression and Node staging			
	Positive	Negative	Total
N staging			
0	16 (72.73%)	6 (27.27%)	22 (100.00%)
1	8 (88.89%)	1 (11.11%)	9 (100.00%)
2	4 (80.00%)	1 (20.00%)	5 (100.00%)
Total	28 (77.78%)	8 (22.22%)	36 (100.00%)
[1]Fisher's exact test			

On comparing The surgical resection cases with lymph node status and KRAS expression, 12/14 cases with positive lymph nodes (N1 + N2 stage) showed positive KRAS expression. 2/14 cases with positive lymph nodes had negative KRAS expression. 16/22 cases with no lymph node status had positive KRAS expression and 6/22 cases had no expression. Statistically, no significant association was found between KRAS expression and Tumor bud score (p= 0.8).

21. Positive BRAF immunohistochemical expression

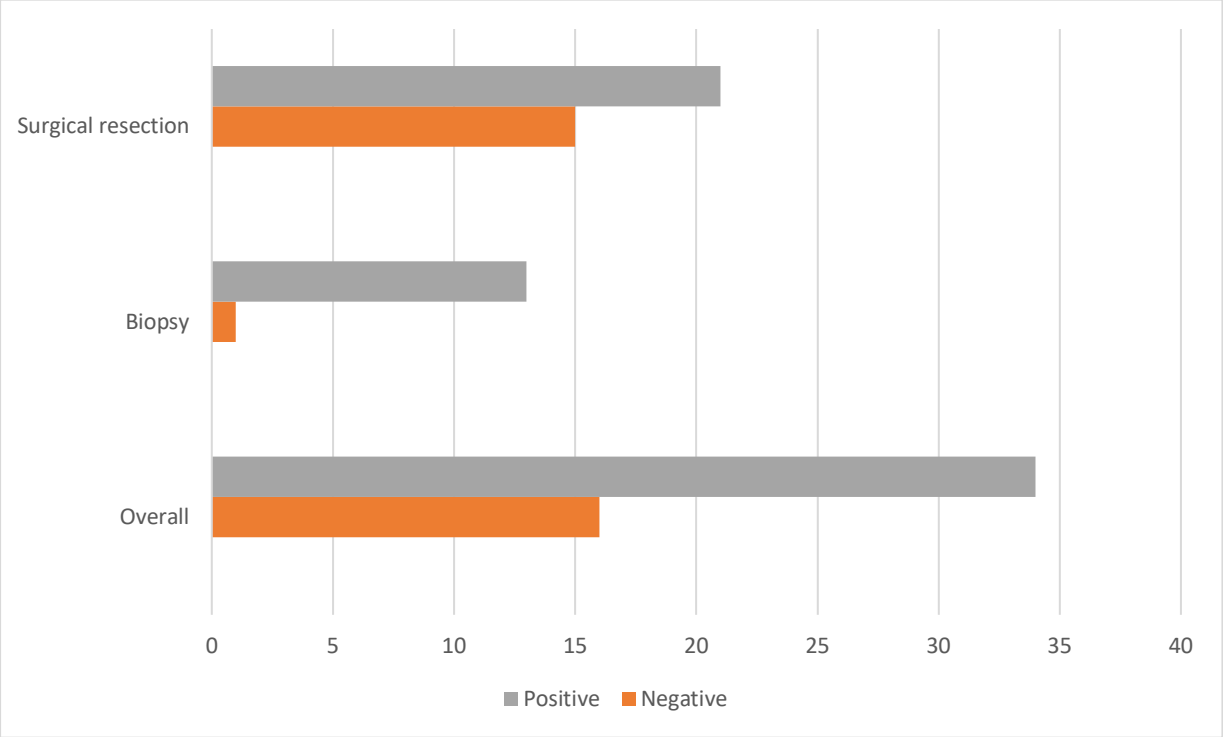

Fig 26. Showing BRAF immunostaining of biopsy and surgical resection cases

A total 50 cases were analysed for BRAF expression. Out of 36 surgical resection cases 21/ 36 cases showed positive expression. 13/14 biopsy cases showed positive expression. Overall 34/50 cases showed positive BRAF status.

22. Grading of BRAF expression in Biopsy and Surgical resection cases

Table 20. Grading of BRAF expression in biopsy and surgical resection cases			
Grade BRAF	Overall, N = 50[1]	Biopsy, N = 14[1]	Surgical resection, N = 36[1]
0	10 (20.00%)	1 (7.14%)	9 (25.00%)
1	6 (12.00%)	0 (0.00%)	6 (16.67%)
2	14 (28.00%)	2 (14.29%)	12 (33.33%)
3	20 (40.00%)	11 (78.57%)	9 (25.00%)

On comparing the grading of BRAF expression in both biopsy and resection cases, Grade 0 was seen in 1/14 biopsies and 9/ 36 surgical resection cases. No biopsy case showed Grade 1 expression. 6/36 surgical resection cases had Grade 1 expression. 2/14 biopsy cases showed Grade 2 expression. 12/36 surgical resection cases showed Grade 2 expression. Maximum cases for biopsy and surgical resection specimens showed Grade 3 expression. A statistically significant association was found (p= 0.008).

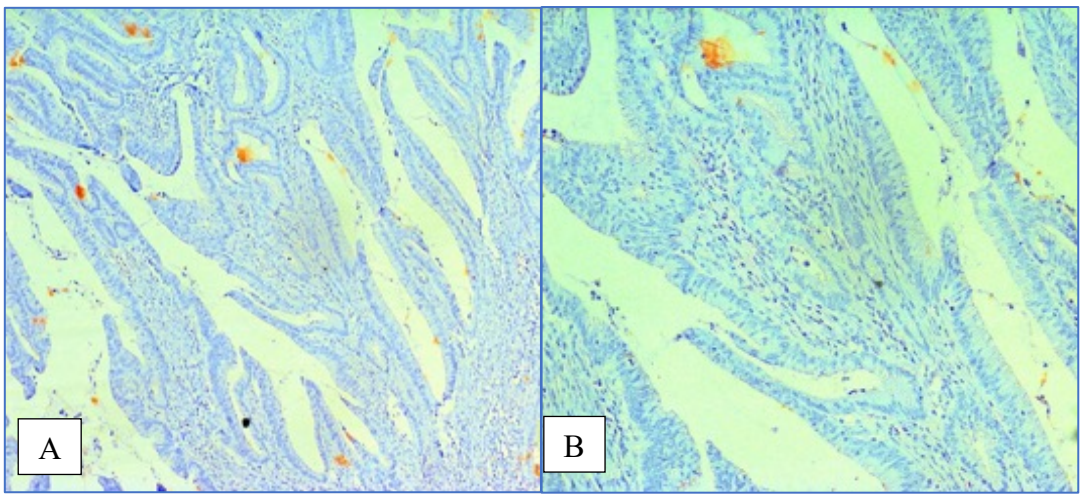

Fig 27. Showing BRAF immunostaining of Grade 0 where no cells show positive staining; A) 10X & B) 20Xrespectively

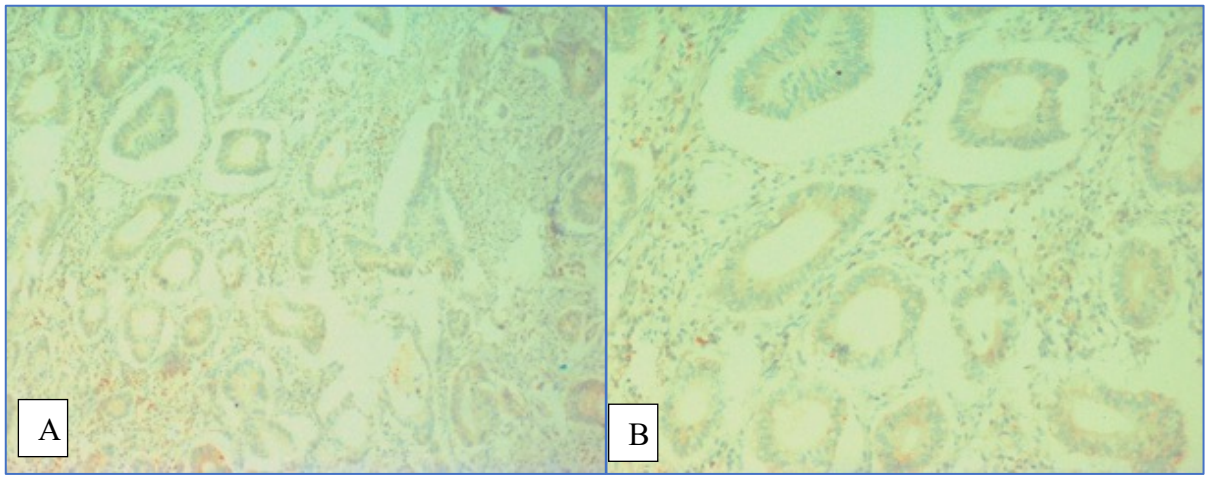

Fig 28. Showing BRAF immunostaining of Grade 1 where weak cytoplasmic staining is seen in 10-33 % of cells; A) 10X & B) 20X.

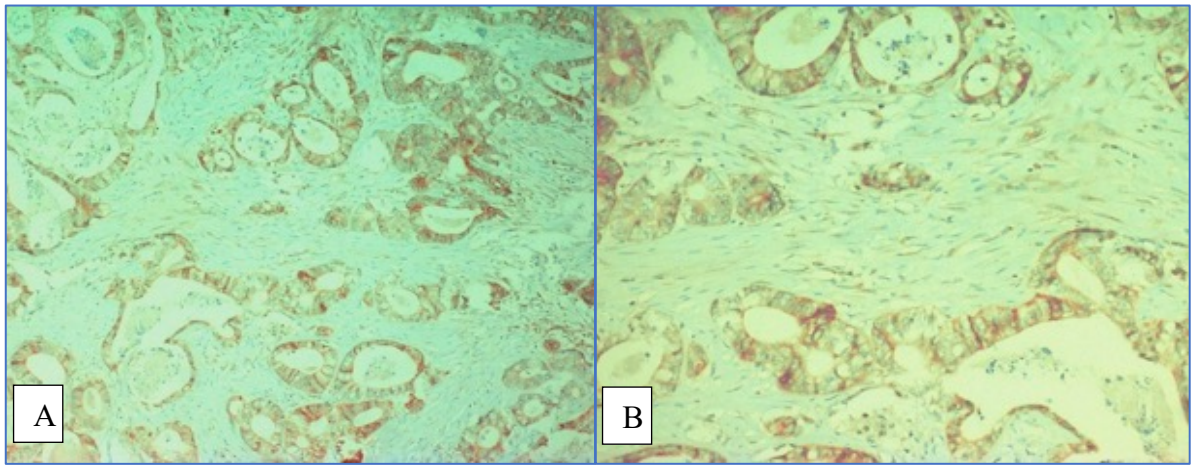

Fig 29. Showing BRAF immunostaining of Grade 2 where moderate intensity cytoplasmic staining is seen in 33-66 % of cells; A) 10X & B) 20X respectively

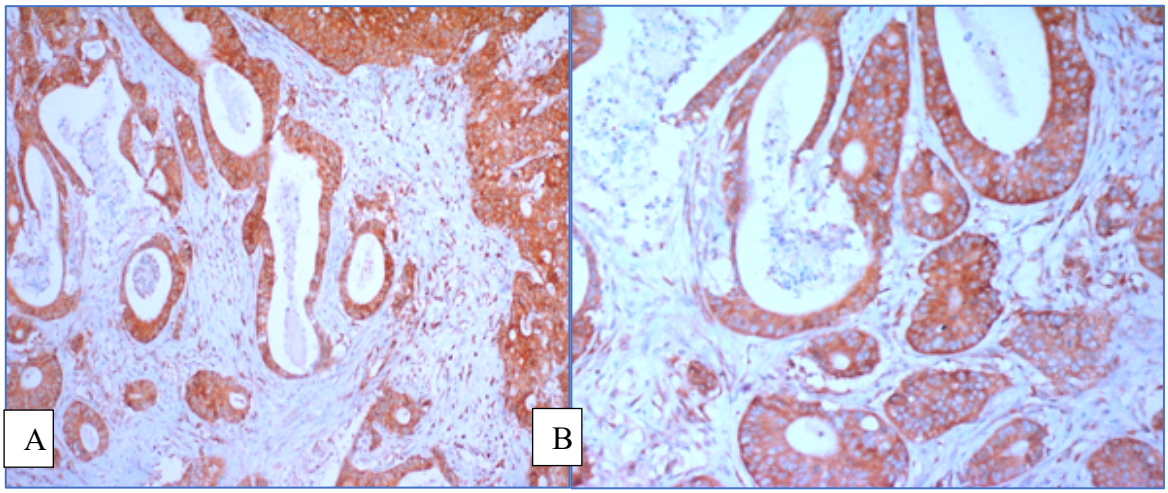

Fig 30. Showing BRAF immunostaining of Grade 3 where strong intensity cytoplasmic staining is seen in > 66 % of cells; A) 10X & B) 20X respectively

Out of a total 36 resection cases, 5 cases were mucinous adenocarcinoma. Two cases were BRAF positive and three are BRAF**.** 1 Signet ring cell carcinoma was BRAF positive.

23. Grading of BRAF immunohistochemical expression and Histological grade

Table 21. BRAF immunohistochemical expression and Histological grade					
	BRAF Grade				
	0	1	2	3	Total
Histological Grade					
Well differentiated	4 (20.00%)	2 (10.00%)	8 (40.00%)	6 (30.00%)	20 (100.00%)
Moderately differentiated	5 (16.00%)	4 (12.00%)	5 (16.00%)	14 (56.00%)	25 (100.00%)
Poorly differentiated	2 (66.67%)	0 (0.00%)	1 (33.33%)	0 (0.00%)	3 (100.00%)
Total	10 (20.00%)	6 (12.00%)	14 (28.00%)	20 (40.00%)	50 (100.00%)
[1]Fisher's exact test					

On comparing the grade of BRAF expression and histological grade in both surgical resection and biopsy cases, no statistically significant association was found between histological type and BRAF positivity (p= 0.10).

24. BRAF immunohistochemical expression and Lymphovascular invasion

Table 22. BRAF immunohistochemical expression and Lymphovascular invasion			
	Grade-BRAF		
	Positive	Negative	Total
Lymphovascular Invasion			
Present	7 (70.00%)	3 (30.00%)	10 (100.00%)
Absent	27 (67.50%)	13 (32.50%)	40 (100.00%)
Total	34 (68.00%)	16 (32.00%)	50 (100.00%)
[1]Fisher's exact test			

On comparing the BRAF expression in two broad categories i.e. positive and negative expression with lymphovascular invasion, 7/ 34 cases with positive BRAF expression showed lymphovascular expression. 27/34 cases with positive BRAF expression did not have lymphovascular invasion. 3/16 cases with negative BRAF expression had lymphovascular invasion. 13/16 cases with negative BRAF expression did not have any evidence of lymphovascular invasion. Statistically no significant association was found between BRAF expression and lymphovascular invasion (p= 0.7).

25. Grading of BRAF immunohistochemical expression and Tumor bud score

Table 23. Grading of BRAF IHC and Tumor bud score					
	Grade-BRAF				
	0	1	2	3	Total
Tumor Bud Score					
Low	6 (17.65%)	1 (2.94%)	11 (32.35%)	16 (47.06%)	34 (100.00%)
Intermediate	4 (44.44%)	2 (22.22%)	2 (22.22%)	1 (11.11%)	9 (100.00%)
High	0 (0.00%)	3 (42.86%)	1 (14.29%)	3 (42.86%)	7 (100.00%)
Total	10 (20.00%)	6 (12.00%)	14 (28.00%)	20 (40.00%)	50 (100.00%)
[1]Fisher's exact test					

On comparing the grade of BRAF immunohistochemical expression and tumor bud score, 3/20 cases had grade 3 expression and high tumor bud score. A statistically significant association was found with $p = 0.010$.

26. Grading of BRAF immunohistochemical expression and T staging

Table 24. Association between Grading of BRAF expression and T staging					
	Grade-BRAF				
	0	1	2	3	Total
T staging					
2	3 (25.00%)	0 (0.00%)	6 (50.00%)	3 (25.00%)	12 (100.00%)
3	5 (22.73%)	6 (27.27%)	5 (22.73%)	6 (27.27%)	22 (100.00%)
4	1 (50.00%)	0 (0.00%)	1 (50.00%)	0 (0.00%)	2 (100.00%)
Total	9 (25.00%)	6 (16.67%)	12 (33.33%)	9 (25.00%)	36 (100.00%)
[1]Fisher's exact test					

For the surgical resection cases(n=36) T staging and Grade of BRAF immunochemical expression association was done. No T1 stage tumors were seen in our study. In T2 stage, 9/12 cases showed positive BRAF expression(Grade 2+ Grade 3). In T3 stage, 11/22 cases had positive BRAF expression. For T4 stage tumors, 1/2 case had positive BRAF expression. Statistically, no significant association was found between BRAF expression and Tumor bud score (p= 0.3).

27. BRAF immunohistochemical expression and Lymph node staging

Table 25. Association between BRAF expression and Lymph node staging			
	BRAF expression		
	Positive	Negative	Total
N staging			
0	15 (68.18%)	7 (31.82%)	22 (100.00%)
1	7 (77.78%)	2 (22.22%)	9 (100.00%)
2	5 (100.00%)	0 (0.00%)	5 (100.00%)
Total	27 (75.00%)	9 (25.00%)	36 (100.00%)
[1]Fisher's exact test			

On comparing the surgical resection cases with lymph node status and BRAF expression, 12/14 cases with positive lymph nodes (N1 + N2 stage) showed positive BRAF expression. 2/14 cases with positive lymph nodes had negative BRAF expression. 15/22 cases with no lymph node status had positive BRAF expression and 7/22 cases had no expression. Statistically, no significant association was found between BRAF expression and Tumor bud score (p= 0.4).

28. Positive and negative for both KRAS and BRAF mutations

Table 26. Association between both KRAS and BRAF positive and negative cases			
	Biopsy	Surgical resection	Total
KRAS/BRAF status			
Negative for both	1 (50.00%)	1 (50.00%)	2 (100.00%)
Positive for both	13 (33.33%)	26 (66.67%)	39 (100.00%)

Out of all 50 cases, 1 biopsy and 1 surgical resection case showed both KRAS and BRAF negative expression. 13 biopsy cases and 26 surgical resection cases showed both KRAS and BRAF positive expression. Statistically no significant association was found with a p value of 0.2.

6. Discussion

In this study, we assessed the histopathological features and Immunohistochemical expression of KRAS and BRAF in colorectal adenocarcinoma. This was done as an observational cross sectional analysis in central India to check KRAS and BRAF expression and compare them with various histopathological features. These gene analyses are gaining attraction in the colorectal adenocarcinoma that may give poor response to the conventional therapies. We also looked at the results and compared it with the available results available in scientific literature where different cut-off have been described for their positivity and negativity for the interpretation of IHC. For both the markers, different staining interpretation protocols had been used in the literature (71)(102).

In the present study a standard widely applicable staining was used for both KRAS and BRAF.

Colorectal adenocarcinoma and Demographic data

Table 27. Comparison of mean age and sex of the patients

Study	Mean age (Overall)	Sex
1. Poornakala et al (103)	58 years	M=332/587 F= 255/587
2. P et al (71)	56 years	M= 37/82 F= 45/82
3. Saxena et al (104)	64 years	M= 44/65 F= 21/65
4. Ye JX et al (105)	65 years	M= 306/535 F= 229/535
5. Present study	48 years	M= 25/50 F= 25/50

In the present study, the mean age was in the fourth decade (48 years) whereas in the other studies, mean age was in the fifth and sixth decade. We had equal number of male and female patients but most of the studies had more male patients than female patients. This may require a larger study group to understand the demographic details.

Site, Histological type and Grade of Colorectal adenocarcinoma

Table 28. Comparison of size histological type and histological grade of Colorectal adenocarcinoma			
Study	Most common site of CRC (Site/ total number of cases)	Most common Histological type (Type/ total number of cases)	Most common Histological grade (Grade/total number of cases)
1. Hugen et al (106)	Colon: 1238/1679 (73.7%)	Adenocarcinoma 1364/1679(81.2%)	-
2. P et al (71)	Colon: 48 (59%)	Adenocarcinoma 61/82 (74%)	Moderately differentiated 59/82 (72%)
3. Alghamdi et al(107)	Colon: 117/194 (60%)	Adenocarcinoma 180/194 (92%)	Low-grade differentiation 137/194 (71%)
4. Midthun et al(108)	Rectum: 2/2 (100%)	Adenocarcinoma 2/2 (100%)	Moderately differentiated 2/2 (100%)
5. Present study	Colon 39/50 (78%)	Adenocarcinoma 41/50 (80%)	Moderately differentiated 27/50 (54%)

In our study, the most common site of colorectal adenocarcinoma is the colon. Even in other similar studies colon was the most common site and the next common is the rectum. Midthun et al. (108)study with two case reports had both arising from rectum. Most common site in the present study and other studies the most common histological type is adenocarcinoma and most common histological grade is moderately differentiated.

Tumor size and lymphovascular invasion

Table 29. Comparison of tumor size and lymphovascular invasion		
Study	**Tumor size (Mean)**	**Lymphovascular invasion (Positive/ total number of cases)**
1. Kornprat et al (109)	4.5 cm	-
2. Liang et al (110)	6.17 cm	122/660 (18%)
3. Dai et al (111)	> 4 cm in 2197/ 4057 (53%)	979/ 4057 (24%)
4. Present study	5.6 cm	10/36 (27%)

In the present study the mean size of the tumor was 5.6 cm. Similar studies showed the tumor size ranging from 4.5 to 6.5 cm. Kornprat et al (109) did not specify the mean size but categorised the patients into two groups where the maximum number of cases was having a tumor size > 4cm. Tumor size considered as an individual prognostic factor as larger tumors had poor prognosis, positive lymph nodes and lymphovascular invasion. In our study, we had 10/36surgical resection cases with lymphovascular invasion. Presence of lymhovascular invasion is also considered as a poor prognostic factor.

Tumor budding

Table 30. Comparison of Tumor bud score		
Study	**Type of specimen**	**Tumor bud score (High grade/ total number of cases)**
1. Zlobec et al (112)	Biopsy	46/133 (34.6%)
2. Blank et al (48)	Surgical resection specimens	18/81 (22%)
3. Kim et al (50)	Surgical resection specimens	35/ 395 (08%)
4. Mehta et al (113)	Surgical resection specimens	17/60 (28.4%)
5. Present study	Biopsy and surgical resection specimens	7/50 (19%)

In our study and other similar studies on tumor bud score and comparing this with other histopathological features like lymph node status, lymphovascular invasion, metastatic deposits etc have found that it is related to high recurrence rate, positive lymph nodes, chemoresistance and poor prognosis. Although H and E staining makes tumour buds visible, but cytokeratin immunohistochemistry can help us better understand them as done in Mehta et al. (113) In the present study we compared tumor budding with lymph node association, and a significant association was found with p= <0.001. A significant association between tumor budding and nodal involvement with p= 0.039 was seen in Mehta et al. (113)Zlobec et al.(112)followed up the biopsy cases with surgical resection cases and the surgical specimens

revealed a strong correlation between the number of tumour buds in the biopsy and the pT stage (P=0.0143), lymph node metastases (P=0.0007), lymphovascular invasion (P=0.0065). These results show tumor budding is associated with poor prognosis and can also be considered as an independent prognostic factors.

KRAS mutation in colon cancer by various methods of testing

Table 31. Comparison of various methods of KRAS mutation

Study	**Method of KRAS mutation analysis**	**Colorectal adenocarcinoma cases with KRAS mutation (Positive for KRAS mutation/ total number of cases)**
1. Alghamdi et al (107)	Real-time PCR	97/194 (50%)
2. Novrial et al (114)	IHC	14/45 (31%)
3. Elsabah et al (70)	IHC	11/26 (42%)
4. Dinu et al (5)	PCR and Pyrosequencing	22/58 (45%)
5. Present study	IHC	36/50 (72%)

In our study we studied the immunohistochemical expression of KRAS mutation and we got positive expression in 36/50 cases (72%). Most of the studies performed mutation analysis for KARS using PCR and pyrosquencing. Alghamdi et al.(107) used real-time PCR to detect KRAS mutation in various exons and had a trend toward right-sided tumours also had

peritoneal metastases. Novrial et al.(114) used IHC for KRAS mutation and did not reveal any significance with age tumor staging and tumor differentiation. Elsabah et al. (70) also used IHC similar to our study for KRAS mutation and did not show any correlation between age and sex.

In our study, positive KRAS mutation was seen mostly in moderately differentiated cases. Out of the total 27 moderately differentiated cases, 18 cases showed positive expression (Table 12). But no significant association was found between grade and KRAS expression. In other similar studies like Elasabah et al.(70), out of a total 8 moderately differentiated cases, 4 cases showed positive KRAS expression on IHC. No significant difference was found as well ($p= 0.68$). In Alghamdi et al.(107) where KRAS mutation was analysed by PCR, histological grade did not show any significant association with KRAS expression ($p= 0.39$).

KRAS mutation and Tumor staging in colon cancer

Table 32. Association between Tumor staging and KRAS mutation		
Study	**Method of KRAS mutation analysis**	**Association between Tumor staging and KRAS expression (Number of cases in the tumor stage/ KRAS positive expression)**
1. Novrial et al (114)	IHC	Stage 2: 7/14(50%) Stage 3: 7/14 (50%)
2. Kodaz et al(115)	Pyrosequencing	T3 stage: 63/90 (70%) T4 stage: 7/90 (08%)
3. Ogino et al (116)	PCR and pyrosequencing	T3 stage:150/178 (84%) T4 stage: 10/178(5%)
4. Present study	IHC	T3 stage: 19/28 (35%) T4 stage: 1/28 (3%)

When the T3 and T4 stages of colon cancer were compared with KRAS-positive cases, in our study, 19 cases were in the T3 stage and 1 case was in T4 stage out of a total of 28 positive cases for KRAS in surgical resection specimens. Novrial et al.(114) did not specify the T stage of the tumor, they had 14 cases positive for KRAS and 7 cases each was in stage 2 and stage 3. Overall including the present study, no significant association has been found between the tumor stage and KRAS mutation.

KRAS mutation and Tumor bud score

Table 33. Comparison between KRAS mutation and tumor bud score		
Study	**Method of KRAS mutation analysis**	**High tumor bud score and positive KRAS mutation**
1. El Agy et al (117)	Sanger sequencing and Pyrosequencing	17/31 (54%)
2. Present study	IHC	6/36 (16%)

In our study 6 cases showed high tumor bud score in 36 KRAS mutant cases. Not many studies are there to compare KRAS mutation and Tumor budding. El Agy et al. (117) is one study who compared various histological features including tumor budding and KRAS mutation. 54% KRAS mutant cases had high tumor bud score with a significant association of p= 0.02. In the present study we did not get any significant association. More research and studies are required to see the association.

KRAS mutation and Lymphovascular invasion in colon cancer

Table 34. Comparison between KRAS mutation and lymphovascular invasion		
Study	**Method of KRAS mutation analysis**	**No of cases with lymphovascular invasion with KRAS mutation**
1. Kodaz et al (115)	Pyrosequencing	41/ 89 (46%)
2. Guo et al (118)	Gene sequencing	330/851 (47%)
3. Present study	IHC	10/10 (100%)

Not many studies have compared KRAS mutation and lymphovascular invasion status. In the present study, 10 cases had lymphovascular invasion and all cases showed positive KRAS expression. A statistically significant association was found between KRAS expression and lymphovascular invasion (p= 0.045). In Guo et al.(118) KRAS mutation was done by exon sequencing and exon-specific mutation analysis was done and compared with lymphovascular invasion. No significant association was found between the two with p= 0.77. In Kodaz et al.(115) KRAS mutation and lymphovascular invasion was compared. No significant association was found between the two(p= 0.30).

BRAF mutation in colon cancer by various methods

Table 35. Comparison of various methods of BRAF mutation		
Study	**Method of BRAF mutation analysis**	**Colorectal adenocarcinoma cases with BRAF mutation (Positive for BRAF mutation/ total number of cases)**
1. Day et al (119)	a. IHC b. Sanger sequencing	a. 63/ 477(13%) b. 56/477(11%)
2. Rossle et al (120)	IHC	20/264 (7%)
3. Piton et al (14)	IHC	20/30 (66%)
4. Saxena et al (104)	IHC	3/65 (4%)
5. Present study	IHC	34/50 (68%)

In our study, 68% of the cases showed positive BRAF immunohistochemical expression. Most of the studies like Day et al.(119) compared IHC with other techniques like Sanger sequencing and pyrosequencing, and found that IHC method for BRAF V600E has 98.2 % sensitivity and specificity of 98.1 %. Roselle et al.(120) had also done DNA extraction and mutation analysis for the cases for the cases which had positive IHC expression. A sensitivity of 100 % and a specificity of 95.24 % compared with combined both Sanger sequencing and ultradeep sequencing as the “gold standard”. Saxena et al.(104) did IHC on 65 cases and only 3 cases

showed positive IHC expression. A significant association was found between BRAF IHC expression and cases examined in the present study.

BRAF mutation and Tumor staging in colon cancer

Table 36. Comparison between BRAF mutation and Tumor staging		
Study	**Method of BRAF mutation analysis**	**Tumor stage and BRAF mutation**
1. Farina Sarasqueta et al (121)	Real time PCR	T3 stage: 42/69 (60%) T4 stage: 13/69(18%)
2. Saxena et al (104)	IHC	T3 stage: 2/3(66%) T4 stage: 1/3(33%)
3. Kwon et al (122)	IHC	T1-T3= 22/51(43%) T4: 16/51(31%)
4. Day et al (119)	IHC	T3= 23/63(36%) T4= 15/63(23%)
5. Present study	IHC	T3= 11/21(52%) T4= 1/21(4%)

In our study, maximum number of cases were in T3 stage which were BRAF positive. Out of 2 T 4 cases, 1 case showed BRAF positive expression. Farina et al.(121) studied BRAF expression with PCR and had maximum number of cases in T3 which were BAF positive. But no significant association was found between T staging and BRAF expression(p= 0.36). Saxena

et al(104) had only 3 cases with BRAF expression out of them 2 cases were in T 3stage and 1 case in T4 stage. Even in the present study, no significant association was found(p= 0.3).

BRAF mutation and Lymphovascular invasion in colon cancer

Table 37. Comparison between BRAF mutation and Lymphovascular invasion		
Study	**Method of BRAF mutation analysis**	**Lymphovascular invasion and BRAF mutation**
1. Jang et al (123)	Pyrosequencing	7/20 (35%)
2. Kwon et al (122)	IHC	31/51 (60%)
3. Present study	IHC	7/34 (20%)

In our study, 7 cases had lymphovascular invasion out 34 BRAF mutated cases. Jang et al.(123) analysed BRAF mutation by pyrosequencing and compared lymphovascular invasion with BRAF mutated cases. They have a significant association with p= <0.001. In another study, Kwon et al. (122) also had a significant association between BRAF mutated cases analysed by IHC and lymphovascular invasion(p=<0.001). In the present study we did not find any significant association between the two (p=0.03).

BRAF mutation and Tumor bud score

Table 38. Comparison between BRAF mutation and Tumor staging		
Study	**Method of BRAF mutation analysis**	**High tumor bud score and positive BRAF mutation**
1. Pai et al (124)	PCR	8/20 (40%)
2. Present study	IHC	4/24 (16%)

In the present study, a high tumor bud score was seen in 4 cases out of 24 BRAF-positive colorectal adenocarcinoma cases. In one study, Pai et al.(124) analysed tumor bud score in BRAF-positive colorectal adenocarcinoma cases and 8 cases out of 20 cases had high tumor bud score, no significant association was found (p=0.07).

Positive and negative for both KRAS and BRAF mutations

Table 39. Comparison between cases with both KRAS and BRAF mutation		
Study	**Method of analysis (Both KRAS and BRAF)**	**Cases with both KRAS and BRAF mutation**
1. P et al (71)	IHC	Positive correlation seen between KRAS and BRAF expression. The data was not statistically significant.
2. Sarasqueta et al(121)	Real-time PCR	No cases had both KRAS and BRAF mutation.
3. Dvorak et al(125)	IHC	No cases showed BRAF IHC expression with KRAS mutation
4. Present study	IHC	38/50 (76%)

In the present study, 76% of the cases showed both KRAS and BRAF IHC expression. P et al (71) did a similar study of KRAS and BRAF IHC expression although no number of cases with both KRAS and BRAF expression was given, a positive correlation seen between KRAS and BRAF expression. The data was not statistically significant (p=0.28). In Saraquesta et al. (121) Real time PCR was done and in Dvorak et al. (125) BRAF IHC was done on both KRAS wild type and mutant type. No cases showed both KRAS and BRAF positive expression in these studies. In the present study, further gene sequencing should be done to confirm the KRAS and BRAF mutations.

Summary

This study was conducted to analyse and compare the histomorphological features of colorectal adenocarcinoma along with Immunohistochemical expression of KRAS and BRAF as there is limited existing literature available in India on this topic.

Title of the study: Immunohistochemical expression of KRAS and BRAF in colorectal adenocarcinoma: Study at tertiary care center from central India

The study period was January 2018 to June 2023, done at Department of Pathology and Laboratory Medicine, AIIMS Bhopal using FFPE archival blocks.

Sample size was decided as convenient sampling of 50 cases.

The study was of both retrospective and prospective type, observational cross- sectional study.

The study was started after getting Institutional Human Ethical Committee Approval (LOP No: IHEC-PGR/2021/PG/Jul/12 dated 28-02-2022).

The study has included 36 resection cases and 14 endoscopic biopsies of colorectal carcinomas following the inclusion and exclusion criteria as mentioned in the materials and methods.

The routine light microscopic findings were noted as per the protocol. In the resection studies, we followed the CAP protocol to report colorectal adeno carcinomas, and noted the histological variant, lymphnodal status, and tumor bud score, lymphovascular invasion, TNM staging etc. But in case of Endoscopic biopsies only, histological grading with variant we could able to record.

All the 50 cases including resection cases as well as endoscopic guided biopsies were subjected to immunohisto chemical study using the KRAS Recombinant Mouse Monoclonal Antibody from Invitrogen (Mouse monoclonal clone 9.13) detection of mutated KRAS and BRAF Rabbit Monoclonal Antibody (Rabbit Monoclonal, clone RM8) from Invitrogen; USA for the detection of mutant BRAF V600E mutation. The automated immuno-stainer (VENTANA

Bench Mark XT) was used to immunostain the slides using the Optiview kit in the department of pathology and lab medicine, AIIMS Bhopal.

Statistical Analysis: The data of the patients were collected and an excel sheet was prepared with the required parameters. The data were analysed in R Studio and analysis was done with R language.

- Mean and standard deviation was used for continuous variables. The distribution of continuous variables was assessed using Shapiro-Wilks test and subsequent analyses were done according to the distribution.
- Pearson's Chi-square and Fisher's exact were used for the comparison of categorical variables.
- Probability values up to 0.05 were considered significant.

Colorectal adenocarcinomas is equally distributed among males and females. Most common side was Right and most common site was Caecum. Majority of the cases were noted in fourth decade with mean age at 48yrs. The common histological type seen was Adenocarcinoma with moderate differentiation. Tumor bud score for the resection cases was done using a three-tier system categorised into low, intermediate and high grade. Statistically significant association was found between tumor bud score and lymph node staging($p<0.001$) stating that tumor bud score itself is an independent prognostic factor for poor prognosis.

KRAS IHC was done in all the 50 cases including surgical resection and biopsy cases. The staining was cytoplasmic and the intensity was graded into 0, 1+, 2+ and 3+. 72% of the cases shows KRAS positive expression. No statistical significance was found between KRAS grade and histological type. A statistically significant association was found between KRAS IHC expression and lymphovascular invasion. No statistical significance was found between Tumor stage, lymph node involvement and Tumor bud score when compared with KRAS expression.

BRAF IHC was also done on all 50 cases including surgical resection and biopsy cases. The staining was cytoplasmic and the intensity was graded into 0, 1+, 2+ and 3+. 68% of the cases shows BRAF positive expression. A statistically significant association was seen between grading of BRAF IHC expression and overall surgical resection and biopsy specimens. No statistical significance was found between BRAF grade and histological type. A statistically significant association was found between BRAF IHC expression and Tumor bud score. No statistical significance was found between Tumor stage, lymph node involvement and lymphovascular invasion when compared with BRAF expression.

Conclusion

To conclude,

1.Over all 50 patients were enrolled for the study including 36 surgical resection specimens and 14 biopsies.

2. Majority of cases were seen in 4^{th} decade, with equal male and female patients.

3. Most common histological variant observed was moderately differentiated adenocarcinoma.

4. Among the histological features tumor bud score had shown significant association with lymphnodestatus.

5. IHC expression of KRAS and BRAF, using monoclonal antibodies, on automated immuno-stainer, VENTANA BENCHMARK XT, revealed a positivity of 72% in KRAS and 68% positivity in BRAF.

6. A statistically significant association was found between KRAS IHC expression and lympho-vascular invasion and BRAF IHC expression showed a statistical significant association with tumour bud score

STRENGTHS

1) Only limited studies are done in India regarding the Immunohistochemical expression of KRAS and BRAF in Colorectal adenocarcinoma.

2) Performing IHC with KRAS and BRAF has found to be sensitive and specific compared to Gene sequencing and PCR.

3) Assessing the tumor bud score and grading it has shown that it is a very important prognostic factor in Colorectal adenocarcinoma along with other histomorphological features,

LIMITATION

Limitations in the present study are, firstly, the sample size was small. No follow up was done in the present study. There were a few cases of mucinous and signet ring adenocarcinoma.

Although IHC for both KRAS and BRAF can be used as a diagnostic method for the mutations, DNA sequencing is considered as a gold standard technique.

In present study we only performed immunohistochemical expression of KRAS and BRAF. However, genetic mutational analysis was not done to confirm the IHC expression due to financial and time constraints. As this molecular work was not within the scope of the present study.

References:

1. Neoplasia In. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran Pathologic Basis of Disease, Professional Edition E-Book. Elsevier Health Sciences; 2014. 246 p.

2. Douaiher J, Ravipati A, Grams B, Chowdhury S, Alatise O, Are C. Colorectal cancer-global burden, trends, and geographical variations. J Surg Oncol. 2017;115(5):619–30.

3. Mohandas KM, Desai DC. Epidemiology of digestive tract cancers in India. V. Large and small bowel. Indian J Gastroenterol Off J Indian Soc Gastroenterol. 1999;18(3):118–21.

4. Blanco-Calvo M, Concha Á, Figueroa A, Garrido F, Valladares-Ayerbes M. Colorectal Cancer Classification and Cell Heterogeneity: A Systems Oncology Approach. Int J Mol Sci. 2015 15;16(6):13610–32.

5. Dinu D, Dobre M, Panaitescu E, Bîrlă R, Iosif C, Hoara P, et al. Prognostic significance of KRAS gene mutations in colorectal cancer--preliminary study. J Med Life. 2014;7(4):581–7.

6. Molina-Cerrillo J, San Román M, Pozas J, Alonso-Gordoa T, Pozas M, Conde E, et al. BRAF Mutated Colorectal Cancer: New Treatment Approaches. Cancers. 2020 14;12(6):1571.

7. Cejas P, López-Gómez M, Aguayo C, Madero R, Carpeño J de C, Belda-Iniesta C, et al. KRAS Mutations in Primary Colorectal Cancer Tumors and Related Metastases: A Potential Role in Prediction of Lung Metastasis. PLOS ONE. 2009 18;4(12):e8199.

8. Colorectal Cancer Screening - NCI [Internet]. 2023 [cited 2023 Oct 3]. Available from: https://www.cancer.gov/types/colorectal/patient/colorectal-screening-pdq

9. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988 1;319(9):525–32.

10. Tanaka H, Deng G, Matsuzaki K, Kakar S, Kim GE, Miura S, et al. BRAF mutation, CpG island methylator phenotype and microsatellite instability occur more frequently and concordantly in mucinous than non-mucinous colorectal cancer. Int J Cancer. 2006 1;118(11):2765–71.

11. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer. 2007;7(4):295–308.

12. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer. 2003;3(6):459–65.

13. Caputo F, Santini C, Bardasi C, Cerma K, Casadei-Gardini A, Spallanzani A, et al. BRAF-Mutated Colorectal Cancer: Clinical and Molecular Insights. Int J Mol Sci. 2019 28;20(21):5369.

14. Piton N, Borrini F, Bolognese A, Lamy A, Sabourin JC. KRAS and BRAF Mutation Detection: Is Immunohistochemistry a Possible Alternative to Molecular Biology in Colorectal Cancer? Gastroenterol Res Pract. 2015;2015:753903.

15. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med. 2004 22;351(4):337–45.

16. Marzouk O, Schofield J. Review of Histopathological and Molecular Prognostic Features in Colorectal Cancer. Cancers. 2011 23;3(2):2767–810.

17. Alzahrani SM, Al Doghaither HA, Al-Ghafari AB. General insight into cancer: An overview of colorectal cancer (Review). Mol Clin Oncol. 2021 1;15(6):1–8.

18. Lotfollahzadeh S, Recio-Boiles A, Cagir B. Colon Cancer. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023].

19. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Przeglad Gastroenterol. 2019;14(2):89–103.

20. Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg. 2009;22(4):191–7.

21. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. Colorectal cancer. Nat Rev Dis Primer. 2015 5;1:15065.

22. Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol. 2012;3(3):153–73.

23. Compton CC, Fielding LP, Burgart LJ, Conley B, Cooper HS, Hamilton SR, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000;124(7):979–94.

24. Blenkinsopp WK, Stewart-Brown S, Blesovsky L, Kearney G, Fielding LP. Histopathology reporting in large bowel cancer. J Clin Pathol. 1981;34(5):509–13.

25. Verhulst J, Ferdinande L, Demetter P, Ceelen W. Mucinous subtype as prognostic factor in colorectal cancer: a systematic review and meta-analysis. J Clin Pathol. 2012;65(5):381–8.

26. Leopoldo S, Lorena B, Cinzia A, Gabriella DC, Angela Luciana B, Renato C, et al. Two subtypes of mucinous adenocarcinoma of the colorectum: clinicopathological and genetic features. Ann Surg Oncol. 2008;15(5):1429–39.

27. Chen JS, Hsieh PS, Chiang JM, Yeh CY, Tsai WS, Tang R, et al. Clinical outcome of signet ring cell carcinoma and mucinous adenocarcinoma of the colon. Chang Gung Med J. 2010;33(1):51–7.

28. Hinoi T, Tani M, Lucas PC, Caca K, Dunn RL, Macri E, et al. Loss of CDX2 expression and microsatellite instability are prominent features of large cell minimally differentiated carcinomas of the colon. Am J Pathol. 2001;159(6):2239–48.

29. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology. 2002;40(5):403–39.

30. Werling RW, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. Am J Surg Pathol. 2003;27(3):303–10.

31. Shih IM, Wang TL, Traverso G, Romans K, Hamilton SR, Ben-Sasson S, et al. Top-down morphogenesis of colorectal tumors. Proc Natl Acad Sci U S A. 2001 27;98(5):2640–5.

32. Tsai CJ, Lu DK. Small colorectal polyps: histopathology and clinical significance. Am J Gastroenterol. 1995;90(6):988–94.

33. Snover DC, Jass JR, Fenoglio-Preiser C, Batts KP. Serrated polyps of the large intestine: a morphologic and molecular review of an evolving concept. Am J Clin Pathol. 2005;124(3):380–91.

34. Blackstone MO, Riddell RH, Rogers BH, Levin B. Dysplasia-associated lesion or mass (DALM) detected by colonoscopy in long-standing ulcerative colitis: an indication for colectomy. Gastroenterology. 1981;80(2):366–74.

35. Half E, Bercovich D, Rozen P. Familial adenomatous polyposis. Orphanet J Rare Dis. 2009 12;4:22.

36. Kopacova M, Tacheci I, Rejchrt S, Bures J. Peutz-Jeghers syndrome: diagnostic and therapeutic approach. World J Gastroenterol. 2009 21;15(43):5397–408.

37. van Lier MGF, Wagner A, Mathus-Vliegen EMH, Kuipers EJ, Steyerberg EW, van Leerdam ME. High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. Am J Gastroenterol. 2010;105(6):1258–64; author reply 1265.

38. Brosens LA, Langeveld D, van Hattem WA, Giardiello FM, Offerhaus GJA. Juvenile polyposis syndrome. World J Gastroenterol. 2011 28;17(44):4839–44.

39. Brosens LAA, van Hattem A, Hylind LM, Iacobuzio-Donahue C, Romans KE, Axilbund J, et al. Risk of colorectal cancer in juvenile polyposis. Gut. 2007;56(7):965–7.

40. Ramirez M, Schierling S, Papaconstantinou HT, Thomas JS. Management of the malignant polyp. Clin Colon Rectal Surg. 2008;21(4):286–90.

41. Ogino S, Goel A. Molecular classification and correlates in colorectal cancer. J Mol Diagn JMD. 2008;10(1):13–27.

42. Snover DC. Update on the serrated pathway to colorectal carcinoma. Hum Pathol. 2011;42(1):1–10.

43. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol. 2010;28(10):1057–68.

44. Yamashita K, Dai T, Dai Y, Yamamoto F, Perucho M. Genetics supersedes epigenetics in colon cancer phenotype. Cancer Cell. 2003;4(2):121–31.

45. Nguyen HT, Duong HQ. The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. Oncol Lett. 2018;16(1):9–18.

46. Zaman A, Bivona TG. Emerging application of genomics-guided therapeutics in personalized lung cancer treatment. Ann Transl Med. 2018;6(9):160.

47. Knudson AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A. 1971;68(4):820–3.

48. Blank A, Schenker C, Dawson H, Beldi G, Zlobec I, Lugli A. Evaluation of Tumor Budding in Primary Colorectal Cancer and Corresponding Liver Metastases Based on H&E and Pancytokeratin Staining. Front Med [Internet]. 2019 [cited 2023 10];6.

49. Cho SJ, Kakar S. Tumor Budding in Colorectal Carcinoma: Translating a Morphologic Score Into Clinically Meaningful Results. Arch Pathol Lab Med. 2018 1;142(8):952–7.

50. Kim J ki, Rhee YY, Bae JM, Kim JH, Koh SJ, Lee HJ, et al. Composite scoring system and optimal tumor budding cut-off number for estimating lymph node metastasis in submucosal colorectal cancer. BMC Cancer. 2022 6;22(1):861.

51. Fuchs TL, Sioson L, Sheen A, Jafari-Nejad K, Renaud CJ, Andrici J, et al. Assessment of Tumor-infiltrating Lymphocytes Using International TILs Working Group (ITWG) System Is a Strong Predictor of Overall Survival in Colorectal Carcinoma: A Study of 1034 Patients. Am J Surg Pathol. 2020;44(4):536–44.

52. Klintrup K, Mäkinen JM, Kauppila S, Väre PO, Melkko J, Tuominen H, et al. Inflammation and prognosis in colorectal cancer. Eur J Cancer Oxf Engl 1990. 2005;41(17):2645–54.

53. Sottoriva A, Kang H, Ma Z, Graham TA, Salomon MP, Zhao J, et al. A Big Bang model of human colorectal tumor growth. Nat Genet. 2015;47(3):209–16.

54. J K, La C, F W, Da G, J G, C C, et al. Integrative, multimodal analysis of glioblastoma using TCGA molecular data, pathology images, and clinical outcomes. IEEE Trans Biomed Eng [Internet]. 2011 Dec [cited 2023];58(12).

55. Jančík S, Drábek J, Radzioch D, Hajdúch M. Clinical Relevance of KRAS in Human Cancers. BioMed Res Int. 2010;2010:e150960.

56. Wennerberg K, Rossman KL, Der CJ. The Ras superfamily at a glance. J Cell Sci. 2005 1;118(5):843–6.

57. Gideon P, John J, Frech M, Lautwein A, Clark R, Scheffler JE, et al. Mutational and Kinetic Analyses of the GTPase-Activating Protein (GAP)-p21 Interaction: The C-Terminal Domain of GAP Is Not Sufficient for Full Activity. Mol Cell Biol. 1992 1;12(5):2050–6.

58. Hancock JF, Prior IA. Electron microscopic imaging of Ras signaling domains. Methods. 2005 1;37(2):165–72.

59. Zhang Z, Wang Y, Vikis HG, Johnson L, Liu G, Li J, et al. Wildtype Kras2 can inhibit lung carcinogenesis in mice. Nat Genet. 2001;29(1):25–33.

60. Kranenburg O. The KRAS oncogene: Past, present, and future. Biochim Biophys Acta BBA - Rev Cancer. 2005 25;1756(2):81–2.

61. Liao J, Wolfman JC, Wolfman A. K-Ras Regulates the Steady-state Expression of Matrix Metalloproteinase 2 in Fibroblasts*. J Biol Chem. 2003 22;278(34):31871–8.

62. Yan Z, Deng X, Chen M, Xu Y, Ahram M, Sloane BF, et al. Oncogenic c-Ki-ras but Not Oncogenic c-Ha-ras Up-regulates CEA Expression and Disrupts Basolateral Polarity in Colon Epithelial Cells*. J Biol Chem. 1997 31;272(44):27902–7.

63. Laghi L, Orbetegli O, Bianchi P, Zerbi A, Di Carlo V, Boland CR, et al. Common occurrence of multiple K-RAS mutations in pancreatic cancers with associated precursor lesions and in biliary cancers. Oncogene. 2002;21(27):4301–6.

64. Huncharek M, Muscat J, Geschwind JF. K-ras oncogene mutation as a prognostic marker in non-small cell lung cancer: a combined analysis of 881 cases. Carcinogenesis. 1999 1;20(8):1507–10.

65. Hwang KT, Kim BH, Oh S, Park SY, Jung J, Kim J, et al. Prognostic Role of KRAS mRNA Expression in Breast Cancer. J Breast Cancer. 2019 2;22(4):548–61.

66. Bazan V, Agnese V, Corsale S, Calò V, Valerio MR, Latteri MA, et al. Specific TP53 and/or Ki-ras mutations as independent predictors of clinical outcome in sporadic colorectal adenocarcinomas: results of a 5-year Gruppo Oncologico dell'Italia Meridionale (GOIM) prospective study. Ann Oncol. 2005 1;16:iv50–5.

67. Brink M, de Goeij AFPM, Weijenberg MP, Roemen GMJM, Lentjes MHFM, Pachen MMM, et al. K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. Carcinogenesis. 2003;24(4):703–10.

68. Chubarov AS, Oscorbin IP, Filipenko ML, Lomzov AA, Pyshnyi DV. Allele-Specific PCR for KRAS Mutation Detection Using Phosphoryl Guanidine Modified Primers. Diagnostics. 2020;10(11):872.

69. Kothari N, Schell MJ, Teer JK, Yeatman T, Shibata D, Kim R. Comparison of KRAS mutation analysis of colorectal cancer samples by standard testing and next-generation sequencing. J Clin Pathol. 2014;67(9):764–7.

70. Elsabah MT, Adel I. Immunohistochemical assay for detection of K-ras protein expression in metastatic colorectal cancer. J Egypt Natl Cancer Inst. 2013 1;25(1):51–6.

71. P K, K G, N G. Immunohistochemical Localization of KRAS and BRAF and its Clinical Utility in Patients with Colorectal Cancer. Colorectal Cancer Open Access [Internet]. 2018 [cited 2023 10];04(01).

72. Rahman S, Garrel S, Gerber M, Maitra R, Goel S. Therapeutic Targets of KRAS in Colorectal Cancer. Cancers. 2021 11;13(24):6233.

73. Lito P, Solomon M, Li LS, Hansen R, Rosen N. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. Science. 2016 5;351(6273):604–8.

74. Canon J, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, et al. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. Nature. 2019;575(7781):217–23.

75. Shaib W, Mahajan R, El-Rayes B. Markers of resistance to anti-EGFR therapy in colorectal cancer. J Gastrointest Oncol [Internet]. 2013 Sep [cited 2023 24];4(3).

76. Loo E, Khalili P, Beuhler K, Siddiqi I, Vasef MA. BRAF V600E Mutation Across Multiple Tumor Types: Correlation Between DNA-based Sequencing and Mutation-specific

Immunohistochemistry. Appl Immunohistochem Mol Morphol AIMM. 2018;26(10):709–13.

77. Matallanas D, Birtwistle M, Romano D, Zebisch A, Rauch J, von Kriegsheim A, et al. Raf family kinases: old dogs have learned new tricks. Genes Cancer. 2011;2(3):232–60.

78. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002 27;417(6892):949–54.

79. Zhang W. BRAF inhibitors: the current and the future. Curr Opin Pharmacol. 2015;23:68–73.

80. Wan PTC, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004 19;116(6):855–67.

81. Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N, et al. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell. 2010 22;140(2):209–21.

82. Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer. 2003;3(1):11–22.

83. Brennan DF, Dar AC, Hertz NT, Chao WCH, Burlingame AL, Shokat KM, et al. A Raf-induced allosteric transition of KSR stimulates phosphorylation of MEK. Nature. 2011 21;472(7343):366–9.

84. Morrison DK, Cutler RE. The complexity of Raf-1 regulation. Curr Opin Cell Biol. 1997;9(2):174–9.

85. Qiu T, Lu H, Guo L, Huang W, Ling Y, Shan L, et al. Detection of BRAF mutation in Chinese tumor patients using a highly sensitive antibody immunohistochemistry assay. Sci Rep. 2015 18;5(1):9211.

86. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EWT, Chang F, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta. 2007;1773(8):1263–84.

87. Sithanandam G, Druck T, Cannizzaro LA, Leuzzi G, Huebner K, Rapp UR. B-raf and a B-raf pseudogene are located on 7q in man. Oncogene. 1992;7(4):795–9.

88. Yao Z, Torres NM, Tao A, Gao Y, Luo L, Li Q, et al. BRAF Mutants Evade ERK-Dependent Feedback by Different Mechanisms that Determine Their Sensitivity to Pharmacologic Inhibition. Cancer Cell. 2015 14;28(3):370–83.

89. Bivona TG. Dampening oncogenic RAS signaling. Science. 2019 22;363(6433):1280–1.

90. Yao Z, Yaeger R, Rodrik-Outmezguine VS, Tao A, Torres NM, Chang MT, et al. Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. Nature. 2017 10;548(7666):234–8.

91. Schindler G, Capper D, Meyer J, Janzarik W, Omran H, Herold-Mende C, et al. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. Acta Neuropathol (Berl). 2011;121(3):397–405.

92. Yan C, Huang M, Li X, Wang T, Ling R. Relationship between BRAF V600E and clinical features in papillary thyroid carcinoma. Endocr Connect. 2019 17;8(7):988–96.

93. Mackiewicz-Wysocka M, Czerwińska P, Filas V, Bogajewska E, Kubicka A, Przybyła A, et al. Oncogenic BRAF mutations and p16 expression in melanocytic nevi and melanoma in the Polish population. Adv Dermatol Allergol Dermatol Alergol. 2017;34(5):490–8.

94. Samowitz WS, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res. 2005 15;65(14):6063–9.

95. Bledsoe JR, Kamionek M, Mino-Kenudson M. BRAF V600E immunohistochemistry is reliable in primary and metastatic colorectal carcinoma regardless of treatment status and shows high intratumoral homogeneity. Am J Surg Pathol. 2014;38(10):1418–28.

96. Zhou S, Sikorski D, Xu H, Zubarev A, Chergui M, Lagacé F, et al. Defining the Criteria for Reflex Testing for BRAF Mutations in Cutaneous Melanoma Patients. Cancers. 2021 10;13(9):2282.

97. Ihle MA, Fassunke J, König K, Grünewald I, Schlaak M, Kreuzberg N, et al. Comparison of high resolution melting analysis, pyrosequencing, next generation sequencing and immunohistochemistry to conventional Sanger sequencing for the detection of p.V600E and non-p.V600E BRAF mutations. BMC Cancer. 201410;14:13.

98. Kuan SF, Navina S, Cressman KL, Pai RK. Immunohistochemical detection of BRAF V600E mutant protein using the VE1 antibody in colorectal carcinoma is highly concordant with molecular testing but requires rigorous antibody optimization. Hum Pathol. 2014;45(3):464–72.

99. Holderfield M, Deuker MM, McCormick F, McMahon M. Targeting RAF kinases for cancer therapy: BRAF-mutated melanoma and beyond. Nat Rev Cancer. 2014;14(7):455–67.

100. Kim KB, Kefford R, Pavlick AC, Infante JR, Ribas A, Sosman JA, et al. Phase II study of the MEK1/MEK2 inhibitor Trametinib in patients with metastatic BRAF-mutant cutaneous melanoma previously treated with or without a BRAF inhibitor. J Clin Oncol Off J Am Soc Clin Oncol. 2013 1;31(4):482–9.

101. Corcoran RB, André T, Atreya CE, Schellens JHM, Yoshino T, Bendell JC, et al. Combined BRAF, EGFR, and MEK Inhibition in Patients with BRAFV600E-Mutant Colorectal Cancer. Cancer Discov. 2018;8(4):428–43.

102. Fisher KE, Cohen C, Siddiqui MT, Palma JF, Lipford EH, Longshore JW. Accurate detection of BRAF p.V600E mutations in challenging melanoma specimens requires stringent immunohistochemistry scoring criteria or sensitive molecular assays. Hum Pathol. 2014;45(11):2281–93.

103. Poornakala S, Prema N. A study of morphological prognostic factors in colorectal cancer and survival analysis [Internet]. Vol. 62, Indian Journal of Pathology and Microbiology. 2019. p. 36–42.

104. Saxena S, Srinivas V, Deb P, Raman DK, Jagani R. A study of BRAF mutation in colorectal carcinoma in Indian population. J Cancer Res Ther. 2018;14(6):1403–6.

105. Ye JX, Liu Y, Qin Y, Zhong HH, Yi WN, Shi XY. KRAS and BRAF gene mutations and DNA mismatch repair status in Chinese colorectal carcinoma patients. World J Gastroenterol. 2015 7;21(5):1595–605.

106. Hugen N, van de Velde CJH, de Wilt JHW, Nagtegaal ID. Metastatic pattern in colorectal cancer is strongly influenced by histological subtype. Ann Oncol. 2014 1;25(3):651–7.

107. Alghamdi M, Alabdullatif N, Al-Rashoud A, Alotaibi J, Alhussaini N, Elsirawani S, et al. KRAS Mutations in Colorectal Cancer: Relationship With Clinicopathological Characteristics and Impact on Clinical Outcomes in Saudi Arabia. Cureus. 2022;14(3):e23656.

108. Midthun L, Shaheen S, Deisch J, Senthil M, Tsai J, Hsueh CT. Concomitant KRAS and BRAF mutations in colorectal cancer. J Gastrointest Oncol. 2019;10(3):577–81.

109. Kornprat P, Pollheimer MJ, Lindtner RA, Schlemmer A, Rehak P, Langner C. Value of tumor size as a prognostic variable in colorectal cancer: a critical reappraisal. Am J Clin Oncol. 2011;34(1):43–9.

110. Liang Y, Li Q, He D, Chen Y, Li J. Tumor size improves the accuracy of the prognostic prediction of T4a stage colon cancer. Sci Rep. 2021 11;11(1):16264.

111. Dai W, Li Y, Meng X, Cai S, Li Q, Cai G. Does tumor size have its prognostic role in colorectal cancer? Re-evaluating its value in colorectal adenocarcinoma with different macroscopic growth pattern. Int J Surg. 2017 1;45:105–12.

112. Zlobec I, Hädrich M, Dawson H, Koelzer VH, Borner M, Mallaev M, et al. Intratumoural budding (ITB) in preoperative biopsies predicts the presence of lymph node and distant metastases in colon and rectal cancer patients. Br J Cancer. 2014 18;110(4):1008–13.

113. Mehta A, Goswami M, Sinha R, Dogra A. Histopathological Significance and Prognostic Impact of Tumor Budding in Colorectal Cancer. Asian Pac J Cancer Prev [Internet]. 2018 Sep [cited 2023 8];19(9).

114. Novrial D, Wijayana KA, Cahyani HK. Immunohistochemistry of KRAS Protein in Colorectal Cancer: In: Proceedings of the 1st Jenderal Soedirman International Medical Conference in conjunction with the 5th Annual Scientific Meeting (Temilnas) Consortium of Biomedical Science Indonesia [Internet]. Purwokerto, Indonesia: SCITEPRESS - Science and Technology Publications; 2020 [cited 2023 9]. p. 47–51.

115. KODAZ H, HACIBEKIROGLU I, ERDOGAN B, TURKMEN E, TOZKIR H, ALBAYRAK D, et al. Association between specific KRAS mutations and the clinicopathological characteristics of colorectal tumors. Mol Clin Oncol. 2015;3(1):179–84.

116. Ogino S, Meyerhardt JA, Irahara N, Niedzwiecki D, Hollis D, Saltz LB, et al. KRAS Mutation in Stage III Colon Cancer and Clinical Outcome Following Intergroup Trial CALGB 89803. Clin Cancer Res Off J Am Assoc Cancer Res. 2009 1;15(23):7322–9.

117. El Agy F, el Bardai S, Bouguenouch L, Lahmidani N, El Abkari M, Benjelloun EB, et al. Prognostic Impact of Tumor Budding on Moroccan Colon Cancer Patients. Int J Surg Oncol. 2022 21;2022:9334570.

118. Guo TA, Wu YC, Tan C, Jin YT, Sheng WQ, Cai SJ, et al. Clinicopathologic features and prognostic value of KRAS, NRAS and BRAF mutations and DNA mismatch repair status: A single-center retrospective study of 1,834 Chinese patients with Stage I-IV colorectal cancer. Int J Cancer. 2019 15;145(6):1625–34.

119. Day F, Muranyi A, Singh S, Shanmugam K, Williams D, Byrne D, et al. A mutant BRAF V600E-specific immunohistochemical assay: correlation with molecular mutation status and clinical outcome in colorectal cancer. Target Oncol. 2015;10(1):99–109.

120. Rössle M, Sigg M, Rüschoff JH, Wild PJ, Moch H, Weber A, et al. Ultra-deep sequencing confirms immunohistochemistry as a highly sensitive and specific method for detecting BRAF V600E mutations in colorectal carcinoma. Virchows Arch. 2013;463(5):623–31.

121. Fariña-Sarasqueta A, Lijnschoten G van, Moerland E, Creemers GJ, Lemmens VEPP, Rutten HJT, et al. The BRAF V600E mutation is an independent prognostic factor for

survival in stage II and stage III colon cancer patients. Ann Oncol. 2010 1;21(12):2396–402.

122. Utility of BRAF VE1 Immunohistochemistry as a Screening Tool for Colorectal Cancer Harboring BRAF V600E Mutation - PMC [Internet].

123. Jang MH, Kim S, Hwang DY, Kim WY, Lim SD, Kim WS, et al. BRAF-Mutated Colorectal Cancer Exhibits Distinct Clinicopathological Features from Wild-Type BRAF-Expressing Cancer Independent of the Microsatellite Instability Status. J Korean Med Sci. 2017;32(1):38–46.

124. Pai RK, Jayachandran P, Koong AC, Chang DT, Kwok S, Ma L, et al. BRAF-mutated, Microsatellite-stable Adenocarcinoma of the Proximal Colon: An Aggressive Adenocarcinoma With Poor Survival, Mucinous Differentiation, and Adverse Morphologic Features. Am J Surg Pathol. 2012;36(5):744–52.

125. Dvorak K, Higgins A, Palting J, Cohen M, Brunhoeber P. Immunohistochemistry with Anti-BRAF V600E (VE1) Mouse Monoclonal Antibody is a Sensitive Method for Detection of the BRAF V600E Mutation in Colon Cancer: Evaluation of 120 Cases with and without KRAS Mutation and Literature Review. Pathol Oncol Res POR. 2019;25(1):349–59.

PATIENT CASE RECORD

Title: Immunohistochemical expression of KRAS and BRAF in colorectal adenocarcinoma: Study at tertiary care center from central India

DEMOGRAPHIC DATA:

Name of the Patient: Histopathology No:

Age / Sex: Registration (UHID) No:

CLINICAL DATA

Clinical History & Examination:

a. Signs and symptoms: **:** Bleeding per rectum, features of obstruction, constipation, abdominal distension, weakness, weight loss
b. Duration:
c. Site of lesion / Radiological features:
d. Description of Growth / lesion :
   Size:
   Type : Ulcerative / Infiltrating / Exophytic / Fungating / Indurative
e. Clinical Diagnosis:
f. Associated Co morbidity:
   Cardiovascular Disease:
   Diabetes Mellitus:
   Previous Cancer or family history for cancer:
   Any Other:
g. Treatment Received:

PATHOLOGICAL DATA

a. Gross examination of the tumor:
b. Type of carcinoma: Colorectal adenocarcinoma / Mucinous adenocarcinoma / Signet-ring cell carcinoma / Serrated adenocarcinoma / / Undifferentiated carcinoma
c. Grade of carcinoma: Well differentiated / Moderately differentiated / Poorly differentiated / Undifferentiated
d. Lymphovascular invasion: Present / Absent

e. Pathological Lymphnode status: Present / Absent

If present;

Lymph Node Number:

Metastasis: Present / Absent

f. Tumor budding: Present / Absent

Low score (0-4) / Intermediate (5-9) / High score (10 or more)

f. Pathological TNM stage:

PARTICIPANT INFORMATION SHEET

Title: Immunohistochemical expression of KRAS and BRAF in colorectal adenocarcinoma: Study at tertiary care center from central India

You are invited to participate in the above mentioned study which will be conducted at AIIMS, Bhopal between the time duration of February 2022 to June 2023. The decision to either participate or not depends entirely on you and it would not affect your treatment or follow up now or in the future. Please read the information carefully before giving your consent. In case the participant still has any inquiries, they can contact medial anytime for the clarifications.

1. Why you are being invited to participate in the study?

   You are being invited as you fulfil the inclusion criteria of this study design. Approximately 50 people diagnosed with the disease and fulfilling the inclusion criteria are expected to participate in the study which shall be conducted at AIIMS, Bhopal.

2. Do I have to agree to being in the study?

   Your participation in the study is completely voluntary. You can agree to participate or decline the invitation. In either situation, your treatment and follow up will not be affected. If you agree to participate in the study, you will have to fill a consent form and sign it. If at any time during the study you want to withdraw your participation, you are completely free to do so.

3. Why is this research being done?

   Colorectal Adenocarcinoma is the third most common cancer worldwide and is one of the leading causes of cancer death in men and women. Study of Immunohistochemical (IHC) expression of KRAS and BRAF that will be applied in this study can be utilized as indicators of disease progression, aggressiveness and can help in management of patients with colorectal carcinomas, thereby reducing mortality.

   What will happen if I agree to participate in the study?

   If you agree to participate in this study, you will have to sign a consent form allowing the researcher to use the blocks and slides for applying Immunohistochemical markers and you will be asked some questions.

4. How long will I be required to take part in the study?

   It’s a single time study. You are otherwise free to deny further participation in the study at any time during the duration of the study without having to give any reason.

5. Does this study involve any risks on my part ?

   No, this study does not involve any invasive tests on your person per se.

6. Are there any benefits for me to be taking part in the study ?

   No financial benefits shall be given for your participation in the study.

7. Are there any costs to be involved in the study ?

   No, you would not be asked to pay for any of the tests we perform in the study.

8. Will my identity be kept confidential ?

   Yes, you may rest assure that your identity will only be known by the above mentioned researchers. Any kind of identifiable information regarding you will not be revealed to any third party unless you give your permission for doing so or if required by the law.

9. What will be done to the information gathered during the study ?

   The information collected during the study will be used for medical reference. The result of the study may be published in medical journal or be shared with another research entity but the identity of the participants will not be revealed. The results of the study will not be shared with any participants.

   Once you have read this information sheet, the researcher will answer any doubts that you might have. If you wish to participate in the proposed study, kindly sign the attached consent form.

   In case of any further queries, you are free to call the researcher at 8106947570 (Dr B Krishna Chandana)

आंशिकसूचनाशीट

शीर्षक: कोलोरेक्टलएडेनोकार्सिनोमामेंकेआरएएसऔरबीआरएफकीइम्यूनोहिस्टोकेमिकलअभिव्यक्ति: मध्यभारतसेतृतीयकदेखभालकेंद्रमेंअध्ययन |

यहअध्ययनएम्सभोपालकेपैथोलॉजीएवंलैबमेडिसिनविभागमेंपीजीरेजिडेंटडॉबीकृष्णचंदनाकेमार्गदर्शनमेंकियाजारहाहै;

डॉईजयशंकर (गाइड), अतिरिक्तप्रोफेसर, पैथोलॉजीऔरलैबमेडिसिनविभाग, एम्सभोपाल

डॉजयकुमारचौरसिया, (सह-गाइड), सहायकप्रोफेसर, पैथोलॉजीऔरलैबमेडिसिनविभाग, एम्सभोपाल;

डॉश्रमणमुखोपाध्याय (सह-गाइड), एसोसिएटप्रोफेसर, पैथोलॉजीऔरलैबमेडिसिनविभाग, एम्सभोपाल;

प्रोनीलकमलकपूर (सह-गाइड), प्रोफेसरऔरप्रमुख, पैथोलॉजीऔरलैबमेडिसिनविभाग, एम्सभोपाल।

डॉभारतीपंड्या (सह-गाइड), एसोसिएटप्रोफेसर, सामान्यसर्जरीविभाग, एम्सभोपाल।

डॉविनय (सह-गाइड),सहयोगी**प्रोफेसर, ऑन्कोसर्जरीविभाग, एम्सभोपाल**

आपकोउपर्युक्तअध्ययनमेंभागलेनेकेलिएआमंत्रितकियाजाताहैजोफरवरी 2022 सेजून 2023 कीअवधिकेबीचएम्स, भोपालमेंआयोजितकियाजाएगा।भागलेनेयानकरनेकानिर्णयपूरीतरहसेआपपरनिर्भरकरताहैऔरयहआपकेउपचारयाअनुवर्तीकार्रवाईकोप्रभावितनहींकरेगा।अभीयाभविष्यमें।कृपयाअपनीसहमतिदेनेसेपहलेजानकारीकोध्यानसेपढ़ें।यदिप्रतिभागीकेपासअभीभीकोईपूछताछहै, तोवेस्पष्टीकरणकेलिएकभीभीमध्यस्थसेसंपर्ककरसकतेहैं।

1. आपकोअध्ययनमेंभागलेनेकेलिएक्योंआमंत्रितकियाजारहाहै?

आपकोआमंत्रितकियाजारहाहैक्योंकिआपइसअध्ययनडिजाइनकेसमावेशनमानदंडकोपूराकरतेहैं।एम्स, भोपालमेंआयोजितकिएजानेवालेअध्ययनमेंबीमारीसेपीड़ितऔरसमावेशनमानदंडोंकोपूराकरनेवालेलगभग 50 लोगोंकेभागलेनेकीउम्मीदहै।

2. क्यामुझेअध्ययनमेंशामिलहोनेकेलिएसहमतहोनाहोगा?

अध्ययनमेंआपकीभागीदारीपूरीतरहसेस्वैच्छिकहै।आपआमंत्रणमेंभागलेनेयाअस्वीकारकरनेकेलिएसहमतहोसकतेहैं।किसीभीस्थितिमें, आपकाउपचारऔरअनुवर्तीकार्रवाईप्रभावितनहींहोगी।यदिआपअध्ययनमेंभागलेनेकेलिएसहमतहैं, तोआपकोएकसहमतिफॉर्मभरनाहोगाऔरउसपरहस्ताक्षरकरनाहोगा।यदिआपअध्ययनकेदौरानकिसीभीसमयअपनीभागीदारीवापसलेनाचाहतेहैं, तोआपऐसाकरनेकेलिएपूरीतरहसेस्वतंत्रहैं।

3. यहशोधक्योंकियाजारहाहै?

कोलोरेक्टलएडेनोकार्सिनोमादुनियाभरमेंतीसरासबसेआमकैंसरहैऔरपुरुषोंऔरमहिलाओंमेंकैंसरसेहोनेवालीमौतोंकेप्रमुखकारणोंमेंसेएकहै ।इसअध्ययनमेंलागूकिएजानेवालेबायोमार्करकीइम्यूनोहिस्टोकेमिकल (आईएचसी) अभिव्यक्तिकाअध्ययनरोगकीप्रगति, आक्रामकताकेसंकेतककेरूपमेंउपयोगकियाजासकताहै, जिससेमृत्युदरकमहोसकतीहै।

यदिमैंअध्ययनमेंभागलेनेकेलिएसहमतहूँतोक्याहोगा?

यदिआपइसअध्ययनमेंभागलेनेकेलिएसहमतहैं, तोआपकोएकसहमतिफॉर्मपरहस्ताक्षरकरनाहोगा, जिसमेंशोधकर्ताकोइम्यूनोहिस्टोकेमिकलमार्करोंकोलागूकरनेकेलिएब्लॉकऔरस्लाइडकाउपयोगकरनेकीअनुमतिहोगीऔरआपसेकुछप्रश्न पूछेजाएंगे।

4. मुझेअध्ययनमेंभागलेनेकेलिएकितनेसमयकीआवश्यकताहोगी?

यहएकबारकाअध्ययनहै।आपअन्यथाबिनाकोईकारणबताएअध्ययनकीअवधिकेदौरानकिसीभीसमयअध्ययनमेंआगेभागलेनेसेइनकारकरने केलिएस्वतंत्रहैं।

5. क्याइसअध्ययनमेंमेरीओरसेकोईजोखिमशामिलहै?

नहीं, इसअध्ययनमेंआपकेव्यक्तिपरकोईभीआक्रामकपरीक्षणशामिलनहींहै।

6. क्याइसअध्ययनमेंभागलेनेसेमुझेकोईलाभहोगा ?

अध्ययनमेंआपकीभागीदारीकेलिएकोईवित्तीयलाभनहींदियाजाएगा।

7. क्याअध्ययनमेंशामिलहोनेकीकोईलागतहै?

नहीं, आपकोअध्ययनमेंकिएजानेवालेकिसीभीपरीक्षणकेलिएभुगतानकरनेकेलिएनहींकहाजाएगा।

8. क्यामेरीपहचानगोपनीयरखीजाएगी?

हां,

आपनिश्चिंतहोसकतेहैंकिआपकीपहचानकेवलउपर्युक्तशोधकर्ताओंद्वाराहीजानीजाएगी।आपकेबारेमेंकिसीभीप्रकारकीपहचानयोग्यजानका रीकिसीतीसरेपक्षकोतबतकप्रकटनहींकीजाएगीजबतककिआपऐसाकरनेकीअनुमतिनहींदेतेयाकानूनद्वाराआवश्यकहोनेपर।

9. अध्ययनकेदौरानएकत्रितकीगईजानकारीकाक्याकियाजाएगा?

अध्ययनकेदौरानएकत्रकीगईजानकारीकाउपयोगचिकित्सासंदर्भकेलिएकियाजाएगा।अध्ययनकापरिणाममेडिकलजर्नलमेंप्रकाशितकिया जासकताहैयाकिसीअन्यशोधसंस्थाकेसाथसाझाकियाजासकताहैलेकिनप्रतिभागियोंकीपहचानकाखुलासानहींकियाजाएगा।अध्ययनकेप रिणामकिसीभीप्रतिभागीकेसाथसाझानहींकिएजाएंगे।

एकबारजबआपइससूचनापत्रकोपढ़लेतेहैं, तोशोधकर्ताआपकेकिसीभीसंदेहकाउत्तरदेगा।यदिआपप्रस्तावितअध्ययनमेंभागलेनाचाहतेहैं, तोकृपयासंलग्नसहमतिफॉर्मपरहस्ताक्षरकरें।

किसीभीअन्यप्रश्नकेमामलेमें, आपशोधकर्ताको 8106947570 (डॉबीकृष्णचंदना) परकॉलकरनेकेलिएस्वतंत्रहैं।

INFORMED CONSENT FORM

Study title: Immunohistochemical expression of KRAS and BRAF in colorectal adenocarcinoma: Study at tertiary care center from central India

Subject's Name:	UHID / CR No:
S/O / D/O:	Date:
Age/Sex:	Address:

I hereby declare that

1. I have been thoroughly explained about the study in my vernacular language by the researcher and all of my queries have been answered by the researcher.
2. I fulfil the criteria required for participation in this study.
3. I have understood the fact that my participation in the study is completely voluntary and my decision would not affect treatment modality, at AIIMS Bhopal, in any way.
4. I have understood that I can withdraw my participation at any time during the study without giving any reasons.
5. I understand that I am not expected to pay for the costs of any of the special tests that the study involves.
6. I also understand that I shall not be given any perks or paid any money for my participation in this study.
7. I understand that my identity will be kept confidential and only the researchers involved with the study directly will be aware of my personal particulars.
8. I allow the researchers to assess my clinical history, records, and research material such as blocks and slides required for the study.
9. I declare that the information I provide to the researchers is true to the best of my knowledge.

Signature of participant:	Signature of witness:
Name of participant:	Name of witness:
Thumb impression:	Thumb impression of witness:

Signature of researcher:

Name of the researcher:

Date / Place:

सूचितसहमतिप्रपत्र

अध्ययनशीर्षक: कोलोरेक्टलएडेनोकार्सिनोमामेंकेआरएएसऔरबीआरएफकीइम्यूनोहिस्टोकेमिकलअभिव्यक्ति: मध्यभारतसेतृतीयकदेखभालकेंद्रमेंअध्ययन

विषयकानाम: यूएचआईडी / सीआरसंख्या:

एस/ओ/डी/ओ: दिनांक:

आयु/लिंग: पता:

मैंएतद्द्वाराघोषणाकरताहूंकि

1. मुझेशोधार्थीद्वारामेरीस्थानीयभाषामेंअध्ययनकेबारेमेंअच्छीतरहसेसमझायागयाहैऔरमेरेसभीप्रश्नोंकाउत्तरशोधकर्ताद्वारादियागयाहै।

2. मैंइसअध्ययनमेंभागलेनेकेलिएआवश्यकमानदंडोंकोपूराकरताहूं।

3. मैंइसतथ्यकोसमझगयाहूंकिअध्ययनमेंमेरीभागीदारीपूरीतरहसेस्वैच्छिकहैऔरमेरेनिर्णयसेएम्सभोपालमेंकिसीभीतरहसेउपचारकेतौर-तरीकोंपरकोईअसरनहींपड़ेगा।

4. मैंसमझगयाहूंकिमैंअध्ययनकेदौरानकिसीभीसमयबिनाकोईकारणबताएअपनीभागीदारीवापसलेसकताहूं।

5. मैंसमझता/समझतीहूंकिमुझसेअध्ययनमेंशामिलकिसीभीविशेषपरीक्षणकीलागतकाभुगतानकरनेकीअपेक्षानहींकीजातीहै।

6. मैंयहभीसमझताहूंकिइसअध्ययनमेंमेरीभागीदारीकेलिएमुझेकोईअनुलाभनहींदियाजाएगायाकोईपैसानहींदियाजाएगा।

7. मैंसमझताहूंकिमेरीपहचानकोगोपनीयरखाजाएगाऔरअध्ययनमेंसीधेतौरपरशामिलशोधकर्ताओंकोहीमेरेव्यक्तिगतविवरणोंकीजानकारीहोगी।

8. मैंशोधकर्ताओंकोअपनेनैदानिक इतिहास, रिकॉर्डऔरशोधसामग्रीजैसेकिअध्ययनकेलिएआवश्यकब्लॉकऔरस्लाइडकाआकलनकरनेकीअनुमतिदेताहूं।

9. मैंघोषणाकरता/करतीहूंकिमेरेद्वाराशोधकर्ताओंकोदीगईजानकारीमेरीजानकारीकेअनुसारसहीहै।

प्रतिभागीकेहस्ताक्षर: गवाहकेहस्ताक्षर:

प्रतिभागीकानाम: गवाहकानाम:

अंगूठेकानिशान: साक्षीकेअंगूठेकानिशान:

शोधकर्ताकेहस्ताक्षर:

शोधकर्ताकानाम:

तिथि / स्थान:दिनांक / स्थान:

Masterchart:

Colorectal adenocarcinoma: Surgical resection cases

Sno	ID	Histo No	Age/ Gender	Type of specimen	Diagnosis	Procedure	Macroscopic Evaluation of Mesorectum	Tumor Site	Histologic Type	Histologic Grade	Tumor Size	Multiple Prim	Tumor Extent	Macroscopic Tumor Perforation	Lymphovascular Invasion	Perineural Invasion	Number of Tumor Buds	Tumor Bud Score	Type of Polyp in	Treatment Effect	Margin Status for Invasive Car	Positive regional lymph nodes	Tumor Deposits	DISTANT METASTASIS	TNM Staging	KRAS % of Positive cells	Intensity	Grade	BRAF% of Intensity		Interpretation
				Colon Cancer cases																	P: Proximal, D: Distal, C: Circumferential										
1		20180813181	51/M	Right hemicolectom	Adenocarcinoma	Right Hemicolectomy	NA	Ileo-cecal junction	Adenocarcinoma	Well differentiated	6.5cm	Nil	Tumor invades through the m	Not identified	Present	Not seen	11	High	NA	NA	P: 8.5 cm, D: 10 cm	One	NA	NA	pT2N1aM0	95	Strong	3	10	1	Negative
2		20180804998	53/F	Right extended hen	Moderately differentiated adenocarcinoma of cae	Right extended hemicolectomy	NA	Caecum	Adenocarcinoma	Moderately differentiated	5 cm	Nil	Tumor invades through the m	Not identified	Not seen	Not seen	3	Low	NA	NA	P: 10 cm, D: 5 cm	Zero	NA	NA	pT2N0Mx	80	Moderate	2	70	1	Negative
3		20180129980	5-40/M	Right Hemicolect	Mucinous adenocarcinoma with neuroblastoma granule	Right Hemicolectomy	NA	Right ascending colon	Mucinous adenocarcinoma	Poorly differentiated	6 cm	Nil	Tumor invades through the m	Not identified	Not seen	Not seen	5	Intermediate	NA	NA	P: 4cm, D: 5cm	Zero	NA	NA	pT2N1aMx	90	Strong	3	0	0	Negative
4		20180304495	58/M	Right Hemicolectom	Well differentiated adenocarcinoma with mucinous	Right hemicolectomy	NA	Caecum	Adenocarcinoma	Well differentiated	6 cm	Nil	Reaching upto serosa	Not identified	Not seen	Not seen	4	Low	NA	NA	P: 3 cm, D: 7.5 cm	Zero	NA	NA	pT2N1aMx	80	Strong	3	70	2	Positive
5		20180826351	51/M	Segment of descen	Moderately Differentiated adenocarcinoma	Segment of descending colon	NA	Colon	Adenocarcinoma	Moderately differentiated	6.5 cm	Nil	Reaching upto serosa	Not identified	Not seen	Not seen	0	Low	NA	NA	P: 5 cm, D: 6.5 cm	Zero	NA	NA	pT2N0Mx	60	Moderate	2	0	0	Negative
6		20180826675	51/F	Right hemicolectom	Mucinous adenocarcinoma	Right hemicolectomy	NA	Ileocaecal junction	Mucinous adenocarcinoma	Moderately differentiated	3 cm	Nil	Tumor invades through the m	Not identified	Not seen	Not seen	7	Intermediate	NA	NA	P: 10 cm, D: 17 cm	One	NA	NA	pT2N1Mx	80	Strong	3	70	1	Negative
7		20180947317	55/2018	Extended Right hen	Adenocarcinoma colon	Extended Right hemicolectomy	NA	Caecum	Adenocarcinoma	Moderate to poorly differentiated	6 cm	Nil	Tumor reaches upto the seri	Not identified	Seen	Not seen	10	High	NA	NA	P: 3.4 cm, D: 10 cm	Zero	NA	NA	pT2N0Mx	90	Moderate	2	90	2	Positive
8		20180928754	55/2018	Right hemicolectom	Well differentiated adenocarcinoma with mucinous	Right hemicolectomy	NA	Caecum	Adenocarcinoma	Well differentiated	6 cm	Nil	Tumor reaches upto the seri	Not identified	Not seen	Not seen	2	Low	NA	NA	P: 3 cm, D: 7.5 cm	Zero	NA	NA	pT2N0Mx	95	Strong	3	95	3	Positive
9		20181173681	53/2018/9	Right hemicolectom	Mucinous Adenocarcinoma	Right hemicolectomy	NA	Ascending colon and caecum	Mucinous adenocarcinoma	Moderately differentiated	5.5 cm	Nil	Tumor reaches upto the seri	Not identified	Seen	Not seen	14	High	NA	NA	P5 cm, D: 6.5 cm	Three	NA	NA	pT2N1bMx	80	Strong	3	90	3	Positive
10		20180803281	53/2018/0	Right hemicolectom	Moderately differentiated adenocarcinoma colon	Right hemicolectomy	NA	Caecum	Adenocarcinoma	Moderately differentiated	5.5 cm	Nil	Tumor reaches upto the seri	Not identified	Seen	Not seen	7	Intermediate	NA	NA	P: 12 cm, D: 5 cm	Four	NA	NA	pT2b_4aM0	80	Strong	2	60	2	Positive
11		20180808447	55/2018/0	Low Anterior Resec	Moderately differentiated adenocarcinoma with mu	Low Anterior Resection	NA	Rectosigmoid	Adenocarcinoma with mucinous	Moderately differentiated	6.5 cm	Nil	Tumor invades through the m	Not identified	Not seen	Not seen	12	High	NA	NA	P: 11 cm, D: 6 cm	Five	NA	NA	pT2b_4aM0	70	Moderate	2	70	1	Negative
12		20180830335	54/2018/0	Low Anterior resect	Well Differentiated adenocarcinoma	Low Anterior resection	NA	Rectosigmoid	Adenocarcinoma	Moderately differentiated	5 cm	Nil	Tumor invades through the m	Not identified	Not seen	Not seen	3	Low	NA	Poor response score 3	P: 10 cm, D: 2 cm;	Zero	NA	NA	pT2N0Mx	80	Moderate	2	70	2	Positive
13		20180918880	51/2018/0	Resection of rectum	Adenocarcinoma rectum	Abdominoperineal resection	NA	Rectum	Mucinous Adenocarcinoma	Moderately to Poorly differentiated	4.5 cm	Nil	Tumor invades through the m	Not identified	Seen	Not seen	8	High	NA	NA	P: 0.7 cm, D: 0.8 cm	Two	Identified	NA	pT3N2bM1c	70	Moderate	2	10	1	Negative
				Liver biopsy	Metastatic deposits in liver								however pointed viscera	peritoneum is free of tumour deposits																	
14		20180825028	55/2018/0	Low Anterior resect	Moderately differentiated adenocarcinoma	Low anterior resection	NA	Rectum	Adenocarcinoma	Moderately differentiated	4cm	Nil	Tumor invades through the m	Not identified	Present	Not seen	2	Low	NA	NA	P: 8cm, D: 1.5cm	Zero	NA	NA	pT2bN0M0	80	Moderate	2	90	2	Positive
15		20180828075	51/2018/0	Resected segment	Signet ring cell carcinoma/poorly cohesive carcinoma	Low anterior resection	NA	Sigmoid colon	Signet ring cell carcinoma	Poorly differentiated carcinoma	4.5 cm	Nil	Tumor invades through the m	Not identified	Present	Present	3	Low	NA	NA	P: 7 cm, D: 3.5 cm;	Zero	NA	NA	pT2N1a1Mx	80	Strong	3	0	0	Negative
16		20181007858	56/2018/2	Right hemicolectom	Moderately differentiated adenocarcinoma	Right hemicolectomy	NA	Caecum	Adenocarcinoma	Moderately differentiated	10cm		Tumor reaching upto the ser	Not identified	Present	Present	6	Intermediate	NA	NA	P: 40 cm, D: 20 cm	Four	NA	NA	pT3N1bM1c	50	Moderate	2	60	2	Positive
17		20180815455	52/2018/2	Resected rectosigm	Well differentiated adenocarcinoma	Resected rectosigmoid junction	NA	Recto-sigmoid	Adenocarcinoma	Well differentiated	4.5 cm	Nil	Tumour invades the muscula	Not identified	Not seen	Not seen	0	Low	NA	NA	P: 3.5 cm, D: 5 cm	None	NA	NA	pT2N0Mx	70	Strong	3	80	2	Positive
18		2.18202E+09	65/2022	Extended hemicole	Well Differentiated adenocarcinoma	Extended hemicolectomy specimen	NA	Ascending Colon	Adenocarcinoma	Well differentiated	6 cm	Nil	Tumor invades muscularis pr	Not identified	Not seen	Not seen	1	Low	NA	NA	P: 15 cm, D: 7 cm	None	NA	NA	pT2N0Mx	80	Weak	1	70	2	Positive
19		2.18202E+09	57/2022	Right hemicolectom	Adenocarcinoma Colon - Well differentiated	Right hemicolectomy	NA	Lower portion of cecum	Adenocarcinoma	Well differentiated	12cm	Nil	Tumor reaching upto the ser	Not identified	Not seen	Not seen	2	Low	NA	NA	P: 15 cm, D: 8 cm	None	Identified	NA	pT4bN0Mx	70	Moderate	2	90	2	Positive
20		2.18202E+09	51/2022	Extended right hen	Mucinous adenocarcinoma	Extended hemicolectomy specimen	NA	Caecum	Adenocarcinoma	Moderately differentiated	9.2 cm	Nil	Tumour is reaching upto the	Not identified	Not seen	Not seen	15	High	NA	NA	P: 15 cm, D: 7 cm	Four	NA	NA	pT2N1aMx	60	Weak	1	70	3	Positive
21		2.18202E+09	52/2022	Right hemicolectom	Adenocarcinoma Colon - Well Differentiated	Right hemicolectomy	NA	Ascending colon	Adenocarcinoma	Well differentiated	8 cm	Nil	Tumor infiltrating the muscle	Not identified	Not seen	Not seen	0	Low	NA	NA	P: 18.5 cm, D: 6.5 cm	None	NA	NA	pT2N0Mx	95	Strong	3	50	2	Positive
22		2.18202E+09	52/2022	Right hemicolectom	Adenocarcinoma Colon - Well differentiated	Right hemicolectomy	NA	Right colon	Adenocarcinoma	Well differentiated	6.5 cm	Nil	Tumor is seen infiltrating the	Not identified	Not seen	Not seen	6	Intermediate	NA	NA	P: 20 cm, D: 20 cm	One	NA	NA	pT2pN1aMx	20	Weak	1	10	1	Negative
23		2.18202E+09	53/2022	Abdominoperineal	Moderately differentiated Adenocarcinoma	Abdomino perineal resection	NA	Rectum	Adenocarcinoma	Moderately differentiated	6 cm	Nil	Invades through muscularis p	Not identified	Not seen	Not seen	19	High	NA	Extensive residual cancer Score-3	P: 15 cm, D: 4 cm Radial margi	None	Present	NA	pT3N1aMx	60	Moderate	2	70	3	Positive
24		2.18202E+09	59/2022	Extended right hen	Well Differentiated adenocarcinoma	Extended right hemicolectomy,	NA	Transverse colon	Adenocarcinoma	Well differentiated	3.5 cm	Nil	Invades through muscularis p	Not identified	Not seen	Not seen	1	Low	NA	NA	P: 17 cm, D: 10 cm	None	NA	NA	pT2N0Mx	70	Moderate	1	0	0	Negative
25		2.18202E+09	54/2022	Segment of bowel	Adenocarcinoma Colon - Well Differentiated	Segment of sigmoid colon and rectum	NA	Rectosigmoid	Adenocarcinoma	Well differentiated	6 cm	Nil	Tumour invades the muscula	Not identified	Not seen	Not seen	1	Low	NA	NA	P: 6 cm, D: 1.5 cm; R: 1.1 cm	None	NA	NA	pT2N0Mx	60	Strong	3	70	2	Positive
26		2.18202E+09	54/2022	Right hemicolectom	Moderately differentiated Adenocarcinoma	Right hemicolectomy specimen	NA	Caecum	Adenocarcinoma	Moderately differentiated	4 cm	Nil	Invades through muscularis p	Not identified	Not seen	Not seen	6	Intermediate	NA	NA	P: 6 cm, D: 21.5 cm	None	NA	NA	pT2N0Mx	0	Absent	0	0	0	Negative
27		2.18202E+09	56/2022	Right hemicolectom	Adenocarcinoma, Well Differentiated	Right hemicolectomy	NA	Hepatic flexure	Adenocarcinoma	Well differentiated	0 cm	Nil	Tumour invades the muscula	Not identified	Not seen	Not seen	2	Low	NA	Extensive residual cancer, (poor response) - score-3	P: 2.2 cm, D: 3.5 cm	None	NA	NA	pT2N0Mx	80	Moderate	1	0	0	Negative
28		2.18202E+09	54/2022	Low anterior resect	Adenocarcinoma, Well Differentiated	Low anterior resection	NA	Rectosigmoid	Adenocarcinoma	Well differentiated	4 cm	Nil	Tumour invades the muscula	Not identified	Not seen	Not seen	0	Low	NA	NA	P: 3.5 cm, D: 8 cm	None	NA	NA	pT2N0Mx	60	Moderate	2	0	0	Negative
29		2.18202E+09	57/2022	Segment of the sig	Adenocarcinoma, Well Differentiated	Segment of the sigmoid colon	NA	Sigmoid colon	Adenocarcinoma	Well differentiated	1 cm	Nil	Tumor invades into muscular	Not identified	Not seen	Not seen	2	Low	NA	Residual cancer with evident tumor regression	P: 5 cm, D: 5 cm	None	NA	NA	pT2N4aMx	80	Moderate	1	0	0	Negative
30		2.18202E+09	56/2022	Right Hemicolectom	Mixed neuroendocrine-non-neuroendocrine neoplas	Right Hemicolectomy	NA	Ascending colon	Mixed neuroendocrine-non-neu	Poorly differentiated	4.5 cm	Nil	Tumor invades through the m	Not identified	Not seen	Not seen	0	Low	NA	NA	P: 21 cm, D: 4 cm	None	NA	NA	pT2N0Mx	0	Absent	0	80	2	Positive
31		2.18202E+09	58/2022	Right hemicolectom	Moderately differentiated Adenocarcinoma	Right Hemicolectomy	NA	Hepatic flexure	Adenocarcinoma	Moderately differentiated	10cm	Nil	Tumor invades visceral perito	Not identified	Not seen	Not seen	6	Intermediate	NA	NA	P: 11 cm, D: 7 cm	None	NA	NA	pT4bN0Mx	70	Weak	1	0	0	Negative
32		2.18202E+09	57/2022	Anterior resection	Moderately differentiated Adenocarcinoma	Anterior resection	NA	Rectosigmoid	Adenocarcinoma	Moderately differentiated	4.5 cm	Nil	Invades into muscularis prop	Not identified	Not seen	Not seen	1	Low	NA	NA	P: 5 cm, D: 2 cm	None	NA	NA	pT2N0Mx	70	Weak	1	70	2	Positive
33		2.18202E+09	54/2022	Extended right hemi	Moderately differentiated adenocarcinoma	Right Hemicolectomy	NA	Caecum abd transverse colon	Adenocarcinoma	Moderately differentiated	6 cm	Nil	Tumor invades into muscular	Not identified	Not seen	Not seen	2	Low	NA	NA	P: 7.5 cm, D: 27 cm	None	NA	NA	pT2m1bM0x	70	Weak	1	60	3	Positive
34		2.18202E+09	55/2022	Left hemicolectom	Mucinous Adenocarcinoma - Moderately differentiat	Left hemicolectomy specimen	NA	Sigmoid colon	Mucinous Adenocarcinoma	Moderately differentiated	6 cm	Nil	Invades through muscularis p	Not identified	Not seen	Not seen	4	Low	NA	NA	P: 1.5 cm, D: 18.5 cm	None	NA	NA	pT2N1aMx	60	Strong	3	70	3	Positive
35		2.18202E+09	56/2022	Resected part of bo	Well differentiated Adenocarcinoma	Right hemicolectomy specimen	NA	Ascending colon	Adenocarcinoma	Well differentiated	9 cm	Nil	Invades into muscularis prop	Not identified	Not seen	Not seen	2	Low	NA	NA	P: 0 cm, D: 16 cm	None	NA	NA	pT2N0Mx	70	Strong	3	90	3	Positive
36		2.18202E+09	58/2022	Excised bowel with	Well differentiated Adenocarcinoma	Right hemicolectomy specimen	NA	Ascending Colon	Adenocarcinoma	Well differentiated	2.5 cm	Nil	Tumor invades the muscularis	Not identified	Not seen	Not seen	3	Low	NA	NA	P: 8 cm, D: 11 cm	None	No	NA	pT2N0M not applicable	60	Moderate	2	60	2	Positive

Colorectal adenocarcinoma: Biopsy cases

S no	ID	S no	Age/ Gender	Procedure	Diagnosis	Specimen Integrity	Tumor Site	Histologic Type	Histologic Grade	Tumor Extent	Lymphovascular Inva	Number of Tumor Buds	Tumor Bud Score	Type of Polyp in which Inv	Margin Status for Invasive Carcinoma	KRAS % of Positive cells	Intensity	Grade	BRAF % of P	Pattern	Grading
1	20190020496	S-394/19	76/F	Colonoscopy guided biopsy	Adenocarcinoma most probably metastatic	Maintained	Sigmoid colon	Adenocarcinoma	Well differentiated	NA	Not seen	2	Low	NA	NA	90	Strong	3	70	3	Positive
2	20190038085	S-897/19	38 Yr/ F	Punch biopsy from anal verge	Mucinous adenocarcinoma	Maintained	Anal Verge	Adenocarcinoma	Moderately differentiated	NA	Not seen	0	Low	NA	NA	80	Strong	3	90	3	Positive
3	20190070407	S-1301/19	51/F	Excision biopsy	Well differentiated adenocarcinoma in ade	Maintained	Rectum	Adenocarcinoma	Well differentiated	Tumor invades through the m	Not seen	0	Low	Adenomatous polyp	NA	100	Strong	3	90	3	Positive
4	20190059675	S-1671/19	53 Yr/ F	Excised Caecal Ulceroproliferative growth	Moderately differentiated Adenocarcinom	Maintained	Caecum	Adenocarcinoma	Moderately differentiated	NA	Not seen	3	Low	NA	NA	60	Weak	1	60	2	Positive
5	20190150658	S-1999/19	66/M	Punch biopsy from rectum	Rectal Adenocarcinoma	Maintained	Rectum	Adenocarcinoma	Moderately differentiated	NA	Not seen	4	Low	NA	NA	80	Strong	3	80	3	Positive
6	20200035023	S-1429/20	60/F	Punch biopsy from rectal lump	Adenocarcinome rectum with adenomatou	Maintained	Rectum	Adenocarcinoma	Well differentiated	NA	Not seen	5	Intermediate	Adenomatous polyp	Free of tumor	30	Weak	1	90	3	Positive
7	20200073261	S-1990/20	25/F	Rectal polyp biopsy	Tubulovillous adenoma with high grade dys	Maintained	Rectum	Adenocarcinoma	Moderately differentiated	NA	Not seen	0	Low	Tubulovillous adenoma	NA	20	Weak	1	80	3	Positive
8	20180065759	S-435/21	42/M	Punch biopsy from anorectal growth	Mucinous Adenocarcinoma	Maintained	Rectum	Mucinous Adeno	Moderately differentiated	NA	Not seen	6	Intermediate	NA	NA	0	Absent	0	0	0	Negative
9	20210118234	S-3973/21	36/M	Biopsy from rectosigmoid of intestine	Adenocarcinoma	Maintained	Rectosigmoid	Adenocarcinoma	Well differentiated	NA	Not seen	0	Low	NA	NA	10	Weak	1	60	2	Positive
10	2.39212E+14	S-782/22	45 Yr/ F	Caecal biopsy	Moderately differentiated Adenocarcinom	Maintained	Caecum	Adenocarcinoma	Moderately differentiated	NA	Not seen	1	Low	NA	NA	40	Weak	1	80	3	Positive
11	2.39212E+14	S-4703/22	62 Y/ M	1. Biopsy from sigmoid colon	Adenocarcinoma with mucinous componen	Maintained	Sigmoid colon	Mucinous Adeno	Moderately differentiated	NA	Not seen	0	Low	NA	NA	90	Strong	3	90	3	Positive
				2. Excised lymph node	Reactive lymph node																
12	2.39212E+14	S-5955/22	33 y/ M	Biopsy from rectum	Well Differentiated Adenocarcinoma	Maintained	Rectum	Adenocarcinoma	Well differentiated	NA	Not seen	0	Low	NA	NA	80	Strong	3	70	3	Positive
13	2.39212E+14	S-6134/22	61 Yr/ F	Endoscopy-guided biopsy from colonic growth	Features are suggestive of Adenocarcinom	Maintained	Colon	Adenocarcinoma	Moderately differentiated	NA	Nil	3	Low	NA	NA	70	Strong	3	60	3	Positive
14	2.39212E+14	S-1951/23	29 Yr/ F	Biopsy from rectal growth	Moderately differentiated adenocarcinom	Maintained	Rectum	Adenocarcinoma	Moderately differentiated	NA	Nil	0	Low	NA	NA	80	Strong	3	80	3	Positive